INTERNATIONAL STANDARD

ISO 23697-1

First edition
2023-02

Water quality — Determination of total bound nitrogen (ST-TN$_b$) in water using small-scale sealed tubes —

Part 1: Dimethylphenol colour reaction

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Reference number
ISO 23697-1:2023(E)

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Published in Switzerland

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Foreword

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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).

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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*.

A list of all parts in the ISO 23697 series can be found on the ISO website.

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.

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Introduction

Total bound nitrogen (ST-TN$_b$) includes all inorganic forms, nitric nitrogen, nitrous nitrogen, ammoniacal nitrogen and all organic forms of nitrogen in a water sample. Nitrate in particular is one of the fundamental nutrients of algae which, in the presence of sufficient quantities of phosphates and other favourable conditions, determine the eutrophication of water. The main sources of nitrogen are both natural and anthropogenic. Of the anthropogenic sources, particular importance can be attributed to domestic wastewater and the use of fertilizers in agriculture. Reduction of nitrogen load is carried out in domestic and industrial wastewater treatment plants through special denitrification processes.

The presence of significant concentrations of nitrogenous substances in water can indicate pollution and pose a risk to health and environment.

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Part 1:
Dimethylphenol colour reaction

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.

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1 Scope

This document specifies a method for the determination of total bound nitrogen (ST-TN$_b$) in water of various origins: groundwater, surface water, and wastewater, in a measuring range of concentration generally between 0,5 mg/l and 220 mg/l of ST-TN$_b$ using the small-scale sealed tube method. Different measuring ranges of small-scale sealed tube methods can be required.

The measuring ranges can vary depending on the type of small-scale sealed tube method of different manufacturers. It is up to the user to choose the small-scale sealed tube with the appropriate application range or to adapt samples with concentrations exceeding the measuring range of a test by preliminary dilution.

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

All small-scale sealed tube methods are based on a heated alkaline potassium persulfate oxidation in a heating block. Different digestion temperatures, 100 °C or 120 °C or 170 °C, and different digestion times are applicable. Dimethylphenol colour reactions are applied, depending on the typical operating procedure of the small-scale sealed tube used, see Clause 9.

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 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 manufacturers with reagent(s) to develop a colour to be read by a photometer or spectrophotometer

3.2
digestion tube
glass tube used to heat and digest samples in an alkaline solution

Note 1 to entry: This tube is capable to keep temperature and pressure generated by the reagents and by the heating block.

3.3
sealed tube-total bound nitrogen
ST-TN$_b$
sum of organically bound and inorganically bound nitrogen present in water or suspended matter measured under the conditions of this method

4 Principle

The sample to be analysed is heated under specific condition (see Clause 9) in a strongly alkaline solution with potassium persulfate. Organic and inorganic nitrogen are oxidized to nitrate.

Nitrate ions react in acidic solution with 2,6-dimethylphenol to give 4-nitro-dimethylphenol which is read at maximum absorption by a photometer or a spectrophotometer.

The method serves to obtain the concentration of total bound nitrogen (ST-TN$_b$) present in the sample.

5 Interferences

Typical interferences are due to metal ions, organic load [chemical oxygen demand (COD)] and chloride. Examples of ion concentrations influencing the analytical results are reported in Table 1.

Every manufacturer of small-scale sealed tube 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 pre-dosed reagents, in the small-scale sealed tube and in the glass digestion tube.

Table 1 — Examples of interfering ions and organic load

Ions	Maximum tolerable concentration mg/l
Na^+, K^+	500
Ag^+	100
Ca^{2+}	50
COD	200
Cl^-	500

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In case that, in the sample, the concentration of interfering ion exceeds the maximum compatible concentration, the user may dilute the sample and proceed with the appropriate measuring range of the small-scale sealed tube.

It is the laboratory's responsibility to identify interferences by, for example, spiking with standards containing known concentration of nitrogen to the sample containing the suspected ion, see Clause 10.

6 Sampling and sample preparation

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

Samples should be analysed as soon as possible and it is not recommended to store samples for longer time (refer to ISO 5667-3).

It is recommended to homogenize the sample to avoid any losses of ST-TN_b. Homogenize the sample for the determination of ST-TN_b using an efficient device (8.8).

Due to the differences in small-scale sealed tube used, 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 Reagents provided by the manufacturers for the small-scale sealed tube method and used to determine ST-TN_b with the dimethylphenol acid colour reaction (see Clause 9).

7.2.1 Sulfuric acid, H_2SO_4.

7.2.2 Phosphoric acid, H_3PO_4.

7.2.3 Sodium hydroxide, NaOH.

7.2.4 Potassium persulfate, $K_2S_2O_8$.

7.2.5 2,6-Dimethylphenol, $C_8H_{10}O$.

7.2.6 Sodium carbonate, Na_2CO_3.

7.2.7 Sodium sulfite, Na_2SO_3.

7.3 Potassium nitrate, KNO_3, salt previously dried at (105 ± 5) °C for 2 h. Potassium nitrate solutions of known concentrations are commercially available and can also be used as reference material.

7.4 Ammonium chloride, NH_4Cl, salt previously dried at (105 ± 5) °C for 2 h. Ammonium chloride solutions of known concentrations are commercially available and can also be used as reference material.

7.5 Sodium ethylenediaminetetraacetic, $C_{10}H_{18}N_2Na_2O_{10}$, salt previously dried at (105 ± 5) °C for 2 h. Solutions of known concentrations are commercially available and can also be used as reference material.

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8 Apparatus

Usual laboratory apparatus and, in particular, the following.

8.1 Photometer or spectrophotometer for reading small-scale sealed tubes.

8.2 Heating block, capable of reaching and maintaining temperatures of (100 ± 5) °C or (120 ± 5) °C with convective heat transfer.

The small-scale sealed tubes shall be inserted into a carousel in direct contact with the metal part of the heating block which has been heated using a resistor. The depth of the holes should be such that adequate heating of the content occurs.

8.3 High temperature heating block, capable of reaching and maintaining temperatures of (170 ± 5) °C with forced air flows to shorten the sample digestion time. The depth of the holes should be such that adequate heating of the content occurs.

8.4 Digestion tubes, with cap, to carry out digestion.

8.5 Small-scale sealed tubes, for different measuring ranges of ST-TN$_b$ concentrations: low, medium and high.

8.6 Calibrated pipettes/micropipettes of various volumes.

8.7 Drying oven, kept at (105 ± 5) °C to dry reference materials for calibration or system checks.

8.8 Homogenization device, for the homogenization of dispersed matter, for example, a suitable rotor/stator homogenizer and a magnetic stirrer.

9 Procedure

9.1 Oxidation and colour reactions principle

Organic and inorganic nitrogen are oxidized to nitrate using potassium persulfate in an alkaline environment while heated by a heating block (8.2 or 8.3) using digestion tubes.

This digestion is slightly different from the one described in ISO 11905-1[2]. The digestion takes place in a digestion tube with digestion reagents (e.g. 7.2.3 and 7.2.4) and added sample.

The digestion temperature as well as the digestion time can vary, according to the different procedure from each manufacturer. It is recommended to follow manufacturer's manual to set temperature and digestion time on the heating block, accordingly.

9.2 Dimethylphenol colour reaction

Digestion temperature shall be set at (120 ± 5) °C or at (100 ± 5) °C on a convective heating block (8.2). The temperature shall be kept for 30 min or 60 min. It is recommended to check digestion times and reaction temperatures on the manufacturer's manual.

Alternative digestion temperature shall be set at (170 ± 5) °C for 15 min on a high temperature heating block (8.3).

Let the digestion tube, with digested sample cool down to room temperature and then transfer into a small-scale sealed tube. Nitrate ions react in acidic solution with 2,6-dimethylphenol to give 4-nitrodimethylphenol which is read at maximum absorption by a photometer or spectrophotometer at a wavelength of (340 ± 5) nm or (350 ± 5) nm or (365 ± 5) nm.

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Sample pH should be between 3 and 9 and at a temperature between 15 °C and 25 °C.

The reagents in the digestion tube are given in Table 2. Sodium hydroxide or sodium carbonate are used to establish alkaline conditions. Some manufacturers additionally apply sodium sulfite as a compensation reagent, in order to remove excess peroxide to prevent too high findings in the colour reaction.

Table 2 — Reagents in the digestion tube given as mass fraction ranges including sample

Reagent name	Mass fraction percentage range
Sodium hydroxide (7.2.3)	0,3 to 2
Sodium carbonate (7.2.6)	0,8 to 1,3
Potassium persulfate (7.2.4)	0,8 to 3
Sodium sulfite (7.2.7)	0,15 to 0,3

The small-scale sealed tubes contain the following reagents including the sample and the reagents given in Table 3.

Table 3 — Reagents in the colour reaction tube given as mass fraction ranges including sample

Reagent name	Mass fraction percentage range
2,6-dimethylphenol (7.2.5)	0,007 to 1
Sulfuric acid (7.2.1)	30 to 50
Phosphoric acid (7.2.2)	25 to 50

10 Quality control

10.1 Verification of the calibration

In general, the photometer or spectrophotometer (8.1) for reading the small-scale sealed tubes is 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.

The acceptance of the calibration should be evaluated using reference material as quality control solution of different concentrations (diluted from 7.3) 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.

To proof the efficiency of digestion, it is possible to use different standards diluted from 7.4 or 7.5.

The user establishes its own reference material levels and acceptability criteria in accordance with the statistical variability provided by the photometer or spectrophotometer manufacturer, or according to current standards or according to its specific requirements. If the data are acceptable, the photometer or spectrophotometer can be used. Otherwise, recalibration or appropriate maintenance and recalibration is 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 an own calibration curve starting from a reference solution (7.3) and diluting 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 with the photometer or spectrophotometer (8.1). It is recommended to store these own calibration curves on the photometer or spectrophotometer, too.

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10.2 System and interferences checks

It is recommended to check the system using a reference material, because of potential variability in manufacturers practices of photometer/spectrophotometer, method followed, reagents used.

It is at least necessary to check the main potential interferences that can be present in the matrix (see Clause 5). It is recommended to run spiked known concentration of reference material or known concentration of interference ion on sample. To check ST-TN$_b$ 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 own criteria concerning quality control frequency.

The acceptance criteria selected shall be compatible with the declared uncertainty.

11 Calculation

The concentration of total bound nitrogen, $C_{\text{ST-TNb}}$, in the tested sample is calculated using Formula (1):

$$C_{\text{ST-TN}_\text{b}} = \rho \cdot d \qquad (1)$$

where

$C_{\text{ST-TNb}}$ is the total bound nitrogen (ST-TN$_b$) concentration expressed in mg/l in the sample;

ρ is the total bound nitrogen (ST-TN$_b$) concentration expressed in mg/l in the sample;

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

If the analysis was performed with a commercially available automated photometer or spectrophotometer, the concentration, $C_{\text{ST-TNb}}$, 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 method applied shall not be used for calculation of results or be reported as such.

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

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

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

$C_{\text{NH4-N}}$ = 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 23697-1:2023;

b) identity of the sample;

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