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

Part 2: Chromotropic acid colour reaction

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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).

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*.

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.

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.

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

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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 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 150 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 test 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 test 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 at 100 °C and different digestion times are applicable. Chromotropic colour reaction is 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
$ST\text{-}TN_b$
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 chromotropic acid which is read at maximum absorption by a photometer or a spectrophotometer.

The method serves to obtain the concentration of total bound nitrogen ($ST\text{-}TN_b$) 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

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 (8.7).

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 chromotropic acid colour reaction (see Clause 9).

7.2.1 Sulfuric acid, H_2SO_4.

7.2.2 Sodium hydroxide, NaOH.

7.2.3 Potassium persulfate, $K_2S_2O_8$.

7.2.4 Chromotropic acid, $C_{10}H_8O_8S_2$.

7.2.5 Sodium metabisulfite, $Na_2S_2O_5$.

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 Ethylenediaminetetraacetic acid disodium salt dihydrate, $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, 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.

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 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 Digestion tubes, with cap, to carry out digestion.

8.4 Small-scale sealed tubes, for different measuring ranges of ST-TN_b concentrations: low, medium and high.

8.5 Calibrated pipettes/micropipettes of various volumes.

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

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

9 Oxidation and colour reactions principle

9.1 General

Organic and inorganic nitrogen are oxidized to nitrate using potassium persulfate in an alkaline environment while heated by a heating block (8.2) 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 7.2.2) and added sample.

It is recommended to follow manufacturer's manual to set temperature and digestion time on the heating block.

9.2 Chromotropic acid colour reaction

Digestion temperature is set at (100 ± 5) °C on a convective heating block (8.2). The temperature shall be kept for 30 min.

Let the digestion tube with digested sample cool down to room temperature. Nitrate ions react in acidic condition with chromotropic acid (7.2.4) in a small-scale sealed tube which is read in a filter photometer at wavelength of (430 ± 5) nm or in a spectrophotometer at wavelength of (410 ± 5) nm.

Sample pH should be kept between 3 and 10 at a temperature between 15 °C and 25 °C.

The reagents in the digestion tube and the small-scale tubes are given in Table 2.

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

Reagent name	Mass fraction percentage range
Sodium hydroxide (7.2.2)	0,3 to 2
Potassium persulfate (7.2.3)	0,8 to 3
Sodium metabisulfite (7.2.5)	0,01 to 5
Chromotropic acid (7.2.4)	0,01 to 1
Sulfuric acid (7.2.1)	50 to 70