

FINAL
DRAFT

INTERNATIONAL
STANDARD

ISO/FDIS
23695

ISO/TC 147/SC 2

Secretariat: DIN

Voting begins on:
2022-10-11

Voting terminates on:
2022-12-06

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 23695:2023

<https://standards.iteh.ai/catalog/standards/sist/09bed68d-df2a-431f-9e3b-7b78180efa58/iso-23695-2023>

RECIPIENTS OF THIS DRAFT ARE INVITED TO SUBMIT, WITH THEIR COMMENTS, NOTIFICATION OF ANY RELEVANT PATENT RIGHTS OF WHICH THEY ARE AWARE AND TO PROVIDE SUPPORTING DOCUMENTATION.

IN ADDITION TO THEIR EVALUATION AS BEING ACCEPTABLE FOR INDUSTRIAL, TECHNOLOGICAL, COMMERCIAL AND USER PURPOSES, DRAFT INTERNATIONAL STANDARDS MAY ON OCCASION HAVE TO BE CONSIDERED IN THE LIGHT OF THEIR POTENTIAL TO BECOME STANDARDS TO WHICH REFERENCE MAY BE MADE IN NATIONAL REGULATIONS.



Reference number
ISO/FDIS 23695:2022(E)

© ISO 2022

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 23695:2023

<https://standards.iteh.ai/catalog/standards/sist/09bed68d-df2a-431f-9e3b-7b78180efa58/iso-23695-2023>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2022

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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.....	3
8 Apparatus.....	4
9 Reaction conditions for the colour reaction.....	4
9.1 General.....	4
9.2 Salicylate based reaction.....	4
9.3 Chlorophenol based reaction.....	5
10 Quality control.....	5
10.1 Verification of the spectrophotometer 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*.

ISO 23695:2023

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.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 23695:2023

<https://standards.iteh.ai/catalog/standards/sist/09bed68d-df2a-431f-9e3b-7b78180efa58/iso-23695-2023>

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 ($\text{NH}_4\text{-N}$) 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 $\text{NH}_4\text{-N}$ or NH_3 or $\text{NH}_3\text{-N}$.

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 ($\text{NH}_4\text{-N}$) concentration ranges from 0,01 mg/l to 1 800 mg/l of $\text{NH}_4\text{-N}$. 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*

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 ions react 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 to be 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 NH₄-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
Cr(VI)	50

Primary amines can react under analysis conditions and lead to overestimated $\text{NH}_4\text{-N}$ 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. The 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 $\text{NH}_4\text{-N}$ 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 $\text{pH} < 2$; 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 $\text{NH}_4\text{-N}$ 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 $\text{NH}_4\text{-N}$ with colour reactions (see [Clause 9](#))

7.3.1 Sodium dichloroisocyanurate, $\text{C}_3\text{Cl}_2\text{N}_3\text{NaO}_3$.

7.3.2 Sodium salicylate, $\text{C}_7\text{H}_5\text{NaO}_3$.

7.3.3 Sodium nitroprusside, $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$.

7.3.4 Chlorophenol, $(\text{Cl})\text{C}_6\text{H}_4\text{OH}$.

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 $\text{NH}_4\text{-N}$ 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 $(105 \pm 5)^\circ\text{C}$ 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 (9.1.2 and 9.1.3).

9.2 Salicylate based reaction

In strongly alkaline solution, adjusted to $\text{pH} > 12$ with sodium hydroxide ([7.3.4](#)) or lithium hydroxide ([7.3.5](#)), 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 (7.3.4) or lithium hydroxide (7.3.5), 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 spectrophotometer 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.