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Rubber, raw natural, and rubber latex, natural — Determination of nitrogen content

Caoutchouc brut naturel et latex de caoutchouc naturel — Dosage de l'azote

Reference number
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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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 2, *Testing and analysis*.

This fourth edition cancels and replaces the third edition (ISO 1656:1996), which has been technically revised.

Rubber, raw natural, and rubber latex, natural — Determination of nitrogen content

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard 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 and to ensure compliance with any national regulatory conditions.

1 Scope

This International Standard specifies a macro-method and a semi-micro method for the determination of nitrogen in raw natural rubber and in natural rubber latex using variants of the Kjeldahl process.

NOTE The determination of nitrogen in natural rubber is usually carried out in order to arrive at an estimate of the protein content. Minor amounts of non-proteinous nitrogen containing constituents are also present. However, in the dry solids prepared from natural rubber latex, these materials can make a substantial contribution to the total nitrogen content.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 123, *Rubber latex — Sampling*

ISO 124, *Latex, rubber — Determination of total solids content*

ISO 1795, *Rubber, raw natural and raw synthetic — Sampling and further preparative procedures*

ISO/TR 9272, *Rubber and rubber products — Determination of precision for test method standards*

3 Principle

A known mass of the sample is digested with a mixture of sulfuric acid, potassium sulfate, and catalytic amounts of copper sulfate and selenium or sodium selenate, thereby converting nitrogen compounds into ammonium hydrogen sulfate from which the ammonia is distilled after making the mixture alkaline.

The distilled ammonia is absorbed either in standard volumetric sulfuric acid solution followed by titration of the excess acid with a standard volumetric base solution or in boric acid solution followed by titration with standard volumetric acid solution (as boric acid is a weak acid, it does not affect the indicator used for this titration).

4 Macro-method

4.1 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

4.1.1 Catalyst mixture or catalyst solution

CAUTION — When working with selenium, avoid breathing vapours and/or contact with skin or clothing. Work only with adequate ventilation.

4.1.1.1 Catalyst mixture

Prepare a finely divided intimate mixture of the following:

- 30 parts by mass of anhydrous potassium sulfate (K_2SO_4);
- four parts by mass of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$);
- one part by mass of selenium powder or two parts by mass of sodium selenate decahydrate ($Na_2SeO_4 \cdot 10H_2O$).

4.1.1.2 Catalyst solution

Dissolve, with heating, the following:

- 110 g of anhydrous potassium sulfate;
- 14,7 g of copper sulfate pentahydrate;
- 3,7 g of selenium or 7,49 g of sodium selenate, in 600 cm^3 of sulfuric acid (4.1.2).

4.1.2 Sulfuric acid, $\rho = 1,84\ g/cm^3$.

4.1.3 Sulfuric acid standard volumetric solution, $c(H_2SO_4) = 0,05\ mol/dm^3$.

4.1.4 Sodium hydroxide standard volumetric solution, $c(NaOH) = 0,1\ mol/dm^3$.

4.1.5 Sodium hydroxide solution, $c(NaOH)$ approximately 10 mol/dm^3.

Dissolve 400 g of solid sodium hydroxide in 600 cm^3 of water and dilute to 1 000 cm^3.

4.1.6 Boric acid solution, $c(H_3BO_3)$ approximately 0,17 mol/dm^3.

Dissolve 10,5 g of solid boric acid in water, warming if necessary, and dilute to 1 000 cm^3 then cool the solution to room temperature.

4.1.7 Mixed indicator solution.

Dissolve 0,1 g of methyl red and 0,05 g of methylene blue in 100 cm^3 of at least 95 % *(V/V)* ethanol.

This indicator might deteriorate during storage and shall therefore be freshly prepared.

4.2 Apparatus

Ordinary laboratory apparatus and Kjeldahl apparatus, with a digestion flask of capacity 800 cm^3.

4.3 Sampling and preparation of test portion

For the determination of nitrogen in raw solid rubber, a test portion shall be taken from the homogenized piece, sampled and prepared in accordance with ISO 1795.

For the determination of nitrogen in latex, a representative portion (as specified in ISO 123) of thoroughly mixed latex containing about 2 g of total solids shall be dried to constant mass, as specified in ISO 124.

4.4 Procedure

4.4.1 Cut about 2 g of the rubber or dried latex, weighed to the nearest 0,5 mg, into small pieces and place in the digestion flask (see 4.2). Add about 13 g of the catalyst mixture (4.1.1.1) and 60 cm^3 of the sulfuric acid (4.1.2) or, alternatively, 65 cm^3 of the catalyst solution (4.1.1.2). Mix the contents of the flask by swirling and then boil gently until the solution is clear. Continue boiling for 1 h.

NOTE Acidic fumes evolved during digestion will be trapped in an alkaline solution and will be neutralized before being discharged.

Allow the digestion flask and its contents to cool to room temperature then cautiously add 200 cm^3 of water and mix by swirling.

Place the receiving flask containing the absorbing solution in position, connect the distillation unit, and then slowly add 150 cm^3 of the sodium hydroxide solution (4.1.5) to the digestion flask from a dropping funnel.

4.4.2 Carry out the absorption and titration of the liberated ammonia by the procedure described in 4.4.2.1 or 4.4.2.2. The temperature of the receiving flask shall be maintained below 30 °C to prevent any loss of ammonia.

NOTE Ensure proper disposal of the selenium-containing waste in the distillation flask.

4.4.2.1 Place 75 cm^3 of water and, by means of a pipette, 25 cm^3 of the standard volumetric sulfuric acid solution (4.1.3) in the receiving flask of the distillation unit together with two drops of mixed indicator solution (4.1.7). Position the receiving flask so that the end of the delivery tube from the condenser dips below the surface of the absorbing solution. While holding the stopper of the digestion flask in place, thoroughly mix the contents by swirling. Immediately commence distillation and continue at a steady rate until 200 cm^3 of distillate have been collected. If the colour of the indicator changes, it indicates alkalinity of the absorbing solution. Discontinue the determination and repeat the procedure using more sulfuric acid or a smaller test portion.

When the distillation is complete (normally, when the volume in the flask reaches about 300 cm^3), titrate the contents with the sodium hydroxide solution (4.1.4), reading the burette to the nearest 0,02 cm^3.

4.4.2.2 Place 100 cm^3 of the boric acid solution (4.1.6) in the receiving flask of the distillation unit with two drops of the mixed indicator solution (4.1.7). Carry out the distillation as described in 4.4.2.1 and titrate the distillate with the sulfuric acid solution (4.1.3), reading the burette to the nearest 0,02 cm^3.

NOTE If the concentrations of the standard volumetric solution used are not exactly as specified in the list of reagents, appropriate corrections are to be made.

4.5 Blank test

In parallel with the determination, carry out a blank test using the same quantities of reagents under the same operating conditions but omitting the test portion.

4.6 Expression of results

4.6.1 When sulfuric acid is used as the absorbing solution as specified in 4.4.2.1, the nitrogen content of the rubber, expressed as a percentage by mass, is given by Formula (1):

$$\frac{\left(V_1 - V_2\right) \times c \times 0{,}014}{m} \times 100 \qquad (1)$$

where

V_1 is the volume, in cubic centimetres, of sodium hydroxide solution (4.1.4) required for the titration;

V_2 is the volume, in cubic centimetres, of sodium hydroxide solution (4.1.4) required for the titration in the blank test;

c is the concentration of sodium hydroxide;

m is the mass, in grams, of the test portion.

Express the results to the nearest 0,01 %.

4.6.2 When boric acid is used as the absorbing solution as specified in 4.4.2.2, the nitrogen content of the rubber, expressed as a percentage by mass, is given by Formula (2):

$$\frac{\left(V_3 - V_4\right) \times c \times 0{,}028}{m} \times 100 \qquad (2)$$

where

V_3 is the volume, in cubic centimetres, of sulfuric acid solution (4.1.3) required for the titration;

V_4 is the volume, in cubic centimetres, of sulfuric acid solution (4.1.3) required for the titration in the blank test;

c is the concentration of sulfuric acid;

m is the mass, in grams, of the test portion.

Express the results to the nearest 0,01 %.

5 Semi-micro method

5.1 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

5.1.1 Catalyst mixture

CAUTION — When working with selenium, avoid breathing vapours and/or contact with skin or clothing. Work only with adequate ventilation.

Prepare a finely divided intimate mixture of the following:

- 30 parts by mass of anhydrous potassium sulfate (K_2SO_4);
- four parts by mass of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$);
- one part by mass of selenium powder or two parts by mass of sodium selenate decahydrate ($Na_2SeO_4 \cdot 10H_2O$).

5.1.2 Sulfuric acid, $\rho = 1{,}84$ g/cm^3.

5.1.3 **Sulfuric acid standard volumetric solution**, *c*(H_2SO_4) 0,01 mol/dm^3.

5.1.4 **Sodium hydroxide solution**, *c*(NaOH) approximately 10 mol/dm^3.

Dissolve 400 g of solid sodium hydroxide in 600 cm^3 of water and dilute to 1 000 cm^3.

5.1.5 **Sodium hydroxide**, standard volumetric solution, *c*(NaOH) = 0,02 mol/dm^3, carbonate-free.

5.1.6 **Boric acid solution**, *c*(H_3BO_3) approximately 0,17 mol/dm^3.

Dissolve 10,5 g of solid boric acid in 200 cm^3 of water, warming if necessary, and dilute to 1 000 cm^3, then cool the solution to room temperature.

5.1.7 **Mixed indicator solution**.

Dissolve 0,1 g of methyl red and 0,05 g of methylene blue in 100 cm^3 of at least 95 % *(V/V)* ethanol.

This indicator might deteriorate during storage and shall therefore be freshly prepared.

5.2 Apparatus

Ordinary laboratory apparatus and the following are to be used.

5.2.1 **Semi-micro Kjeldahl digestion apparatus**.

5.2.1.1 Digestion flasks of capacity 30 cm^3 and 10 cm^3 (for an example of a typical apparatus, see Figure 1, Figure 2, and Figure 3).

5.2.1.2 Automated digestion block (for an example of a typical apparatus, see Figure 10).

5.2.2 Semi-micro Kjeldahl distillation unit, with a condenser tube of silver, borosilicate glass, or tin (for an example, see Figure 4 to Figure 9).

5.2.3 Semi-micro burette of capacity 5 cm^3 or 10 cm^3, graduated in 0, 02 cm^3 divisions.

5.3 Sampling and preparation of test portion

For the determination of nitrogen in raw solid rubber, a test portion shall be taken from the homogenized piece, sampled and prepared in accordance with ISO 1795.

For the determination of nitrogen in latex, a representative portion (as specified in ISO 123) of thoroughly mixed latex containing about 0,1 g of total solids shall be dried to constant mass, as specified in ISO 124.

5.4 Procedure

5.4.1 Weigh to the nearest 0,1 mg, 0,1 g to 0,2 g of the rubber or dried latex and place in a digestion flask (5.2.1.1). Add about 0,65 g of the catalyst mixture (5.1.1) and 3,0 cm^3 of the sulfuric acid (5.1.2) and heat the contents carefully to the boiling point. Continue boiling for a further 30 min after the digest has become clear and green with no yellow tint.

NOTE Acidic fumes evolved during digestion will be trapped in an alkaline solution and will be neutralized before being discharged.

Avoid excess boiling, as indicated by a tendency for the digest to solidify on cooling, since this can lead to loss of nitrogen.

Bring the water in the steam generator of the distillation unit to boil and pass steam through the semi-micro Kjeldahl distillation unit (5.2.2), including the receiving flask, for at least 2 min. The water jacket of the condenser shall be empty of water during the steaming-out operation. Meanwhile, cool the digestion flask to room temperature or below, add 10 cm^3 of water, and immediately transfer the contents to the distillation flask at the conclusion of the steaming-out process. Complete the transfer by rinsing three times with 3 cm^3 portions of water and draining the flask thoroughly after each transfer.

5.4.2 Discard any condensate which has been collected in the receiver and complete the distillation and titration of ammonia by the procedure described in 5.4.2.1 or 5.4.2.2. The temperature of the receiving flask shall be maintained below 30 °C to prevent loss of ammonia.

NOTE Ensure proper disposal of the selenium-containing waste in the distillation flask.

5.4.2.1 Add from the semi-micro burette (5.2.3) to the steamed-out receiver of the distillation apparatus a measured volume of sulfuric acid solution (5.1.3), using at least 5 cm^3 (the exact volume depending on the amount of nitrogen expected), together with two drops of the mixed indicator solution (5.1.7) and about 5 cm^3 of water. Position the receiver so that the end of the delivery tube from the condenser dips below the surface of the acid. It is an advantage to tilt the receiver slightly to gain a greater depth of liquid.

Add approximately 15 cm^3 of the sodium hydroxide solution (5.1.4) to the distillation flask by means of a measuring cylinder and pass steam from the generator through the distillation flask for 10 min to 12 min at such a rate that the final volume of liquid in the receiver is about 70 cm^3. If the colour of the indicator changes, indicating alkalinity of the absorbing solution, discontinue the determination and repeat the procedure using more sulfuric acid or a smaller test portion.

When the distillation is complete, lower the receiving flask until the tip of the condenser is above the level of the acid, continue the distillation for another 1 min and then rinse the tip of the condenser tube with a few cubic centimetres of water which shall be collected in the distillate. Immediately titrate the contents of the receiving flask with the sodium hydroxide solution (5.1.5), reading the burette to the nearest 0,02 cm^3.

5.4.2.2 Place about 10 cm^3 of the boric acid solution (5.1.6) in the steamed-out receiving flask with two drops of the mixed indicator solution (5.1.7). Carry out the distillation as described in 5.4.2.1, but note that, in the presence of boric acid, the indicator colour should change immediately distillation of ammonia commences. Titrate the distillate with sulfuric acid solution (5.1.3), reading the burette to the nearest 0,02 cm^3.

5.5 Blank test

In parallel with the determination, carry out a blank test using the same quantities of reagents under the same operating conditions but omitting the test portion.

5.6 Expression of results

5.6.1 When sulfuric acid is used as the absorbing solution as specified in 5.4.2.1, the nitrogen content of the rubber, expressed as a percentage by mass, is given by Formula (3):

$$\frac{(V_1 - V_2) \times c \times 0{,}014}{m} \times 100 \qquad (3)$$

where

V_1 is the volume, in cubic centimetres, of sodium hydroxide solution (5.1.5) required for the titration;

V_2 is the volume, in cubic centimetres, of sodium hydroxide solution (5.1.5) required for the titration in the blank test;

c is the concentration of sodium hydroxide;

m is the mass, in grams, of the test portion.

Express the results to the nearest 0,01 %.

5.6.2 When boric acid is used as the absorbing solution as specified in 5.4.2.2, the nitrogen content of the rubber, expressed as a percentage by mass, is given by Formula (4):

$$\frac{(V_3 - V_4) \times c \times 0{,}028}{m} \times 100 \qquad (4)$$

where

V_3 is the volume, in cubic centimetres, of sulfuric acid solution (5.1.3) required for the titration;

V_4 is the volume, in cubic centimetres, of sulfuric acid solution (5.1.3) required for the titration in the blank test;

c is the concentration of sulfuric acid;

m is the mass, in grams, of the test portion.

Express the results to the nearest 0,01 %.

6 Precision

See Annex B.

7 Test report

The test report shall include the following information:

a) reference to this International Standard (i.e. ISO 1656) and the method used;

b) all details necessary for the identification of the material tested;

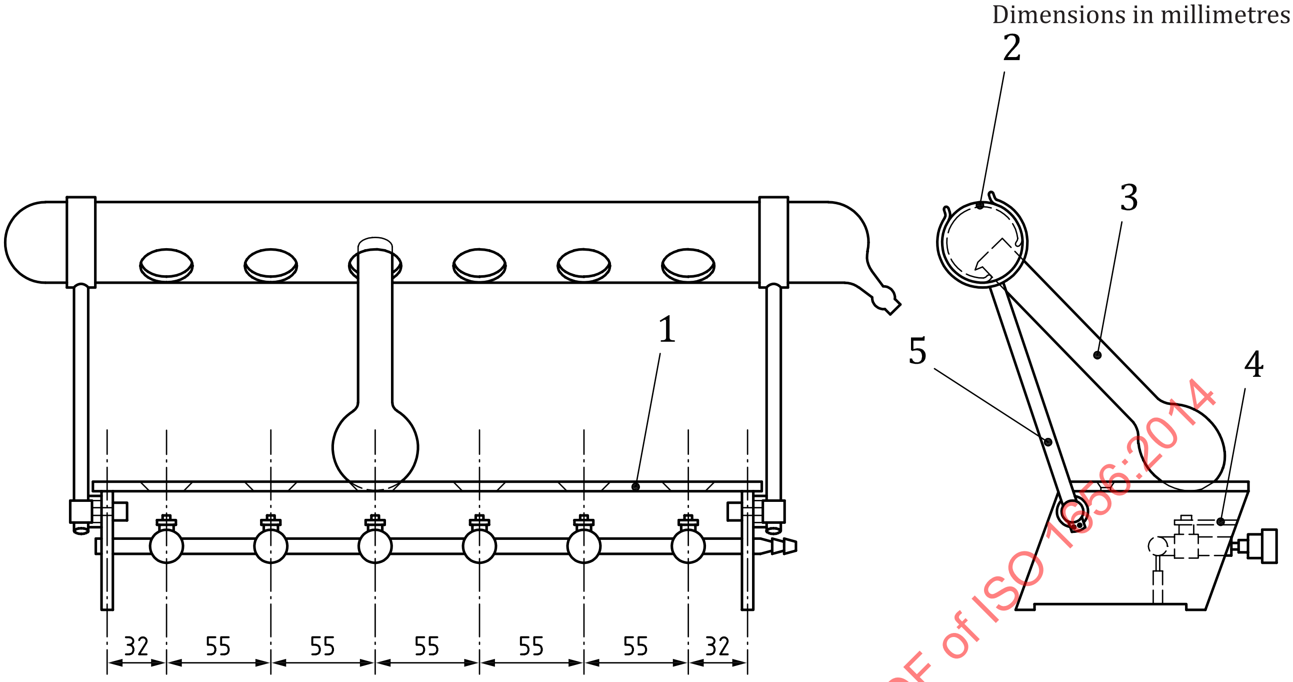
c) results and the units in which they are expressed;

d) any unusual features noted during the determination;

e) any operation not included in this International Standard or in the International Standards to which reference is made and any operation regarded as optional;

f) date of the test.

NOTE If the concentrations of the standard volumetric solutions used are not exactly as specified in the list of reagents, appropriate corrections are to be made.



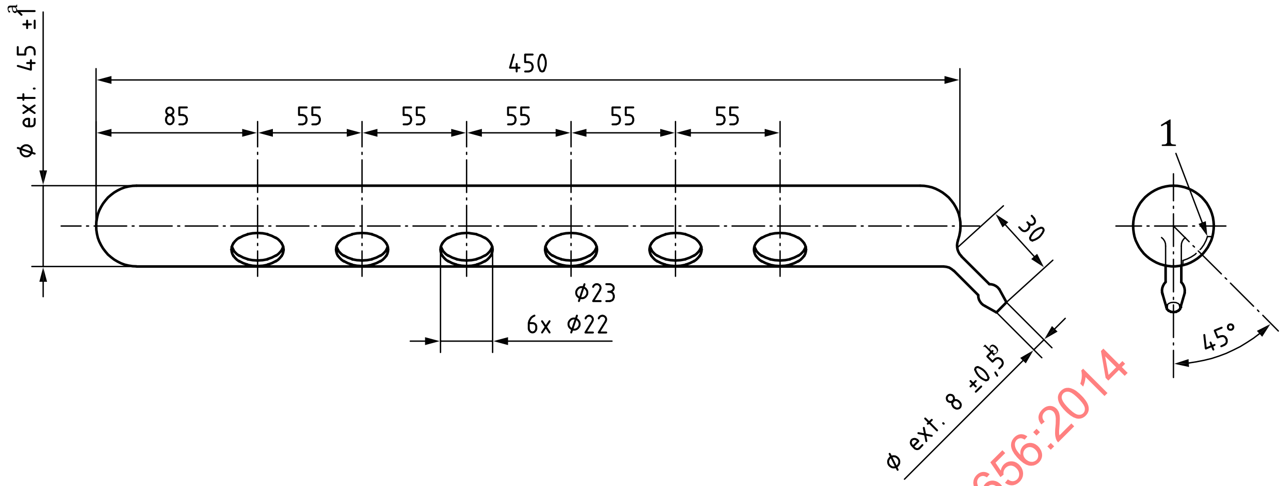
Key

1 shelf made of heat-resistant, thermally insulating material
2 exhaust tube
3 digestion flask
4 micro-burner
5 support rod with adjustment for angle and length

NOTE Where no tolerances are shown, normal working tolerances are allowed.

Figure 1 — Assembly of digestion apparatus for the semi-micro method

Dimensions in millimetres



Key

1 internal flange

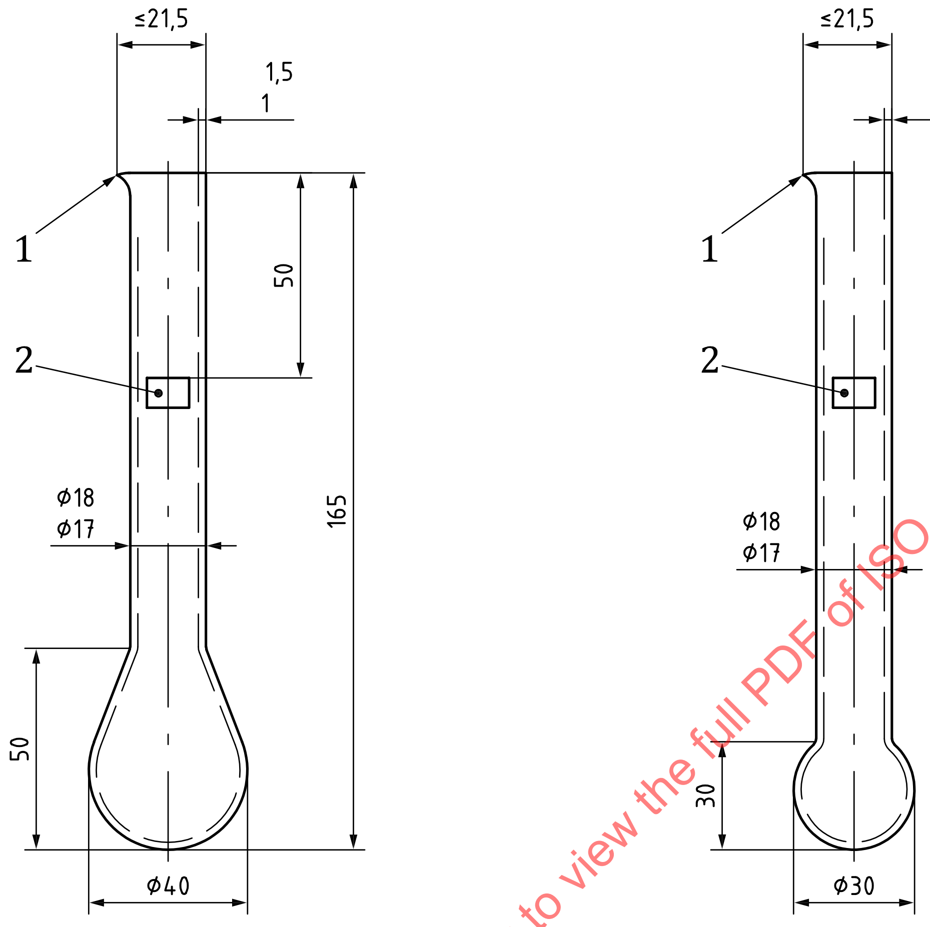
a Wall 1,5 mm to 2,25 mm.

b Wall 1,25 mm to 1,75 mm.

NOTE Where no tolerances are shown, normal working tolerances are allowed.

Figure 2 — Exhaust tube for the semi-micro method

Dimensions in millimetres



a) 30 cm³ flask

b) 10 cm³ flask

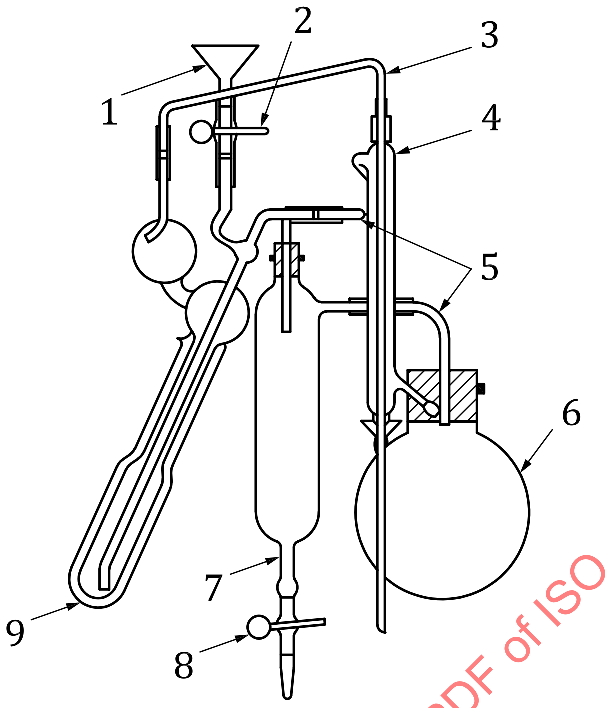
Key

1 spout

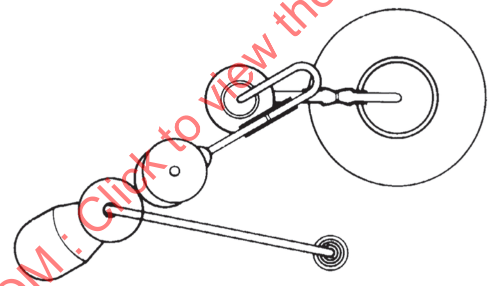
2 labelling badges

NOTE Where no tolerances are shown, normal working tolerances are allowed.

Figure 3 — Digestion flasks for the semi-micro method



Elevation
(Three-quarter view as indicated by plan below)



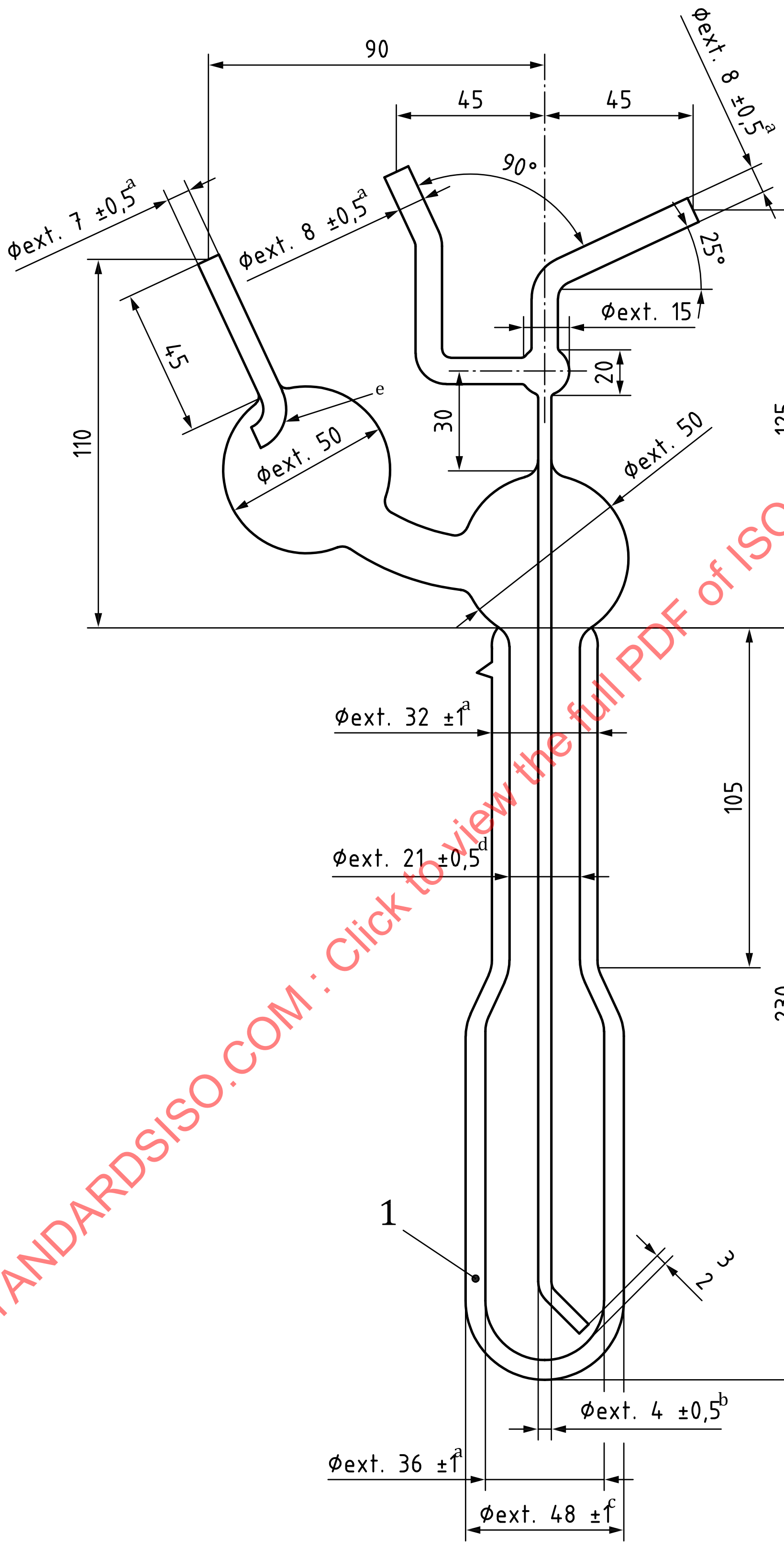
Plan

Key

1	funnel, ⌀ 50mm
2	spring clip
3	condenser tube
4	condenser
5	connecting tubes bent to fit after flasks are in position
6	steam-generating flask (1 dm^3 flask with bolthead)
7	trap
8	spring clip
9	distillation flask

Figure 4 — Assembly of distillation apparatus for the semi-micro method

Dimensions in millimetres



Key

1 jacket highly evacuated

a Wall 1,25 mm to 1,75 mm.

b Wall 0,5 mm to 1 mm.

c Wall 1,5 mm to 2,25 mm.

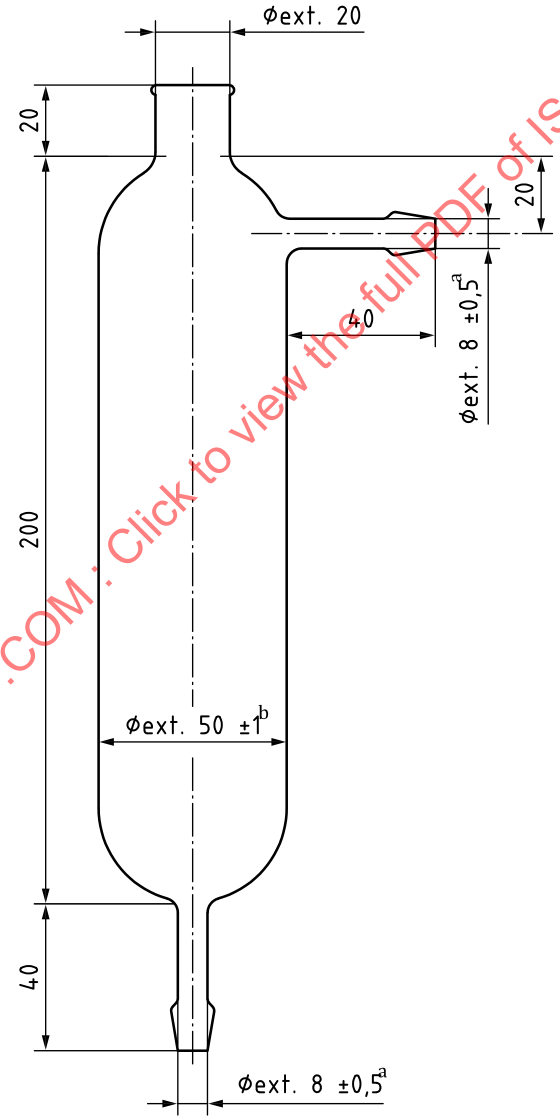
d Wall 1 mm to 1,5 mm.

e Hole ⌀ 3 mm to ⌀ 4 mm.

NOTE Where no tolerances are shown, normal working tolerances are allowed.

Figure 5 — Distillation flask for the semi-micro method

Dimensions in millimetres



Key

a Wall 1,25 mm to 1,75 mm.

b Wall 1,25 mm to 2,25 mm.

NOTE Where no tolerances are shown, normal working tolerances are allowed.

Figure 6 — Trap for the semi-micro method

Dimensions in millimetres

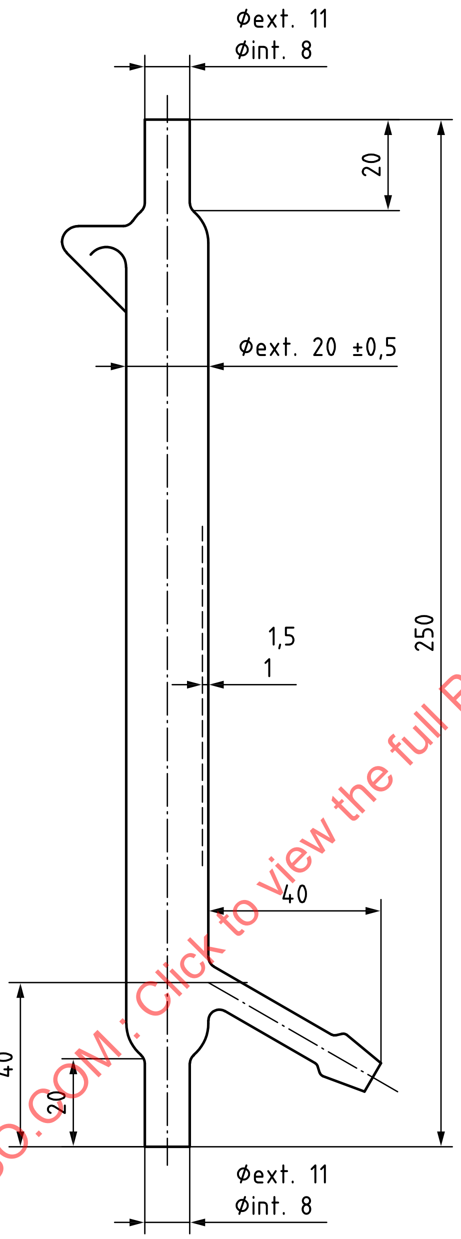
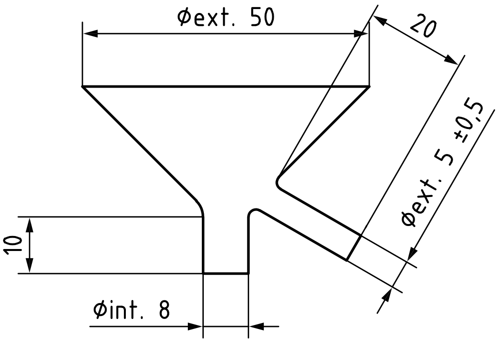


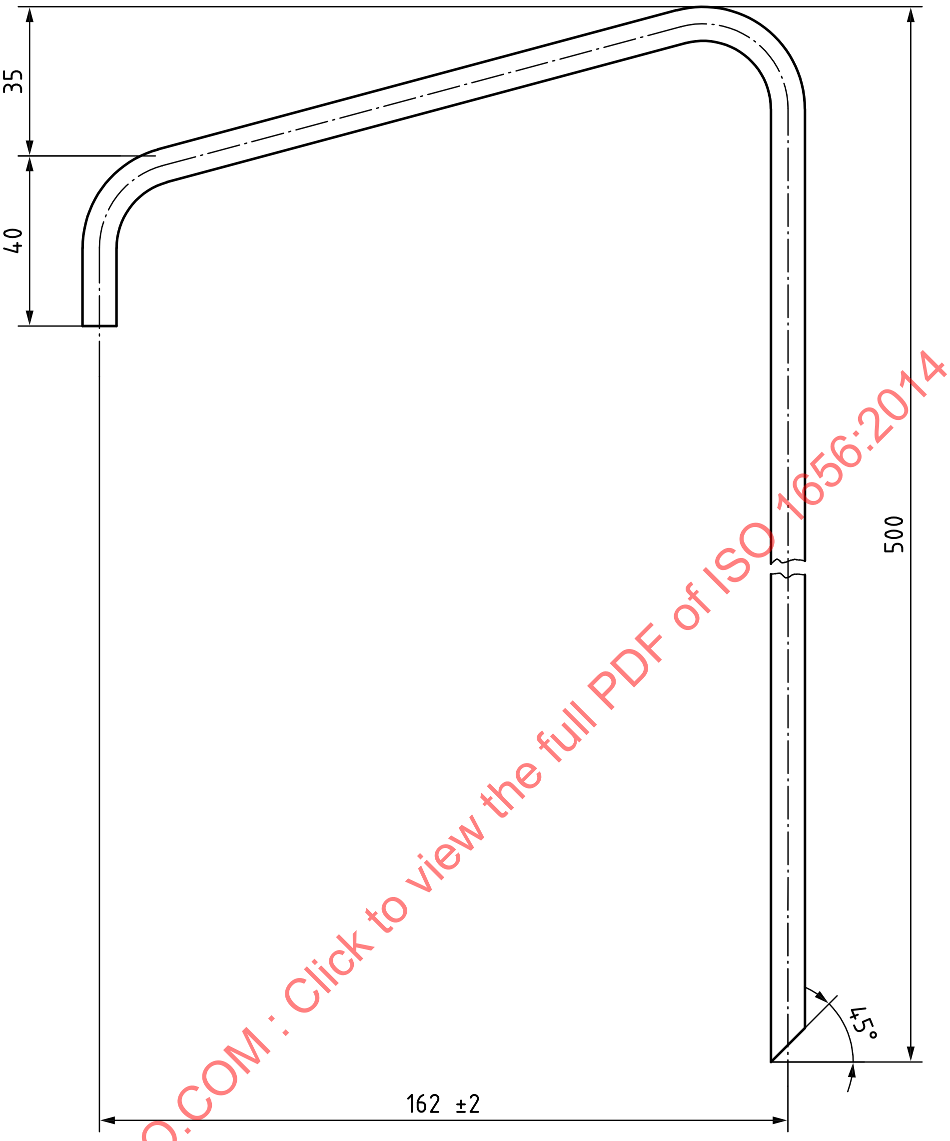
Figure 7 — Condenser jacket for the semi-micro method



NOTE Where no tolerances are shown, normal working tolerances are allowed.

Figure 8 — Drip funnel for the semi-micro method

Dimensions in millimetres



NOTE Where no tolerances are shown, normal working tolerances are allowed.

Figure 9 — Condenser tube for the semi-micro method