

INTERNATIONAL STANDARD

ISO
1656

Third edition
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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
ISO 1656:1996(E)

Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 1656 was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*.

This third edition cancels and replaces the second edition (ISO 1656:1988), which has been technically revised.

Annex A of this International Standard is for information only.

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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 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 1 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, and 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 standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 123:1985, *Rubber latex — Sampling*.

ISO 124:1992, *Rubber latices — Determination of total solids content*.

ISO 1795:1992, *Rubber, raw, natural and synthetic — Sampling and further preparative procedures*.

ISO/TR 9272:1986, *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

- 30 parts by mass of anhydrous potassium sulfate (K_2SO_4);
- 4 parts by mass of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$);
- 1 part by mass of selenium powder, or 2 parts by mass of sodium selenate decahydrate ($Na_2SeO_4 \cdot 10H_2O$).

4.1.1.2 Catalyst solution

Dissolve, with heating,

- 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 cm³ of sulfuric acid (4.1.2).

4.1.2 Sulfuric acid, ρ 1,84 g/cm³.

4.1.3 Sulfuric acid, standard volumetric solution, $c(H_2SO_4) = 0,05$ mol/dm³.

4.1.4 Sodium hydroxide, standard volumetric solution, $c(NaOH) = 0,1$ mol/dm³.

4.1.5 Sodium hydroxide, solution, $c(NaOH) \approx 10$ mol/dm³.

Dissolve 400 g of solid sodium hydroxide in 600 cm³ of water.

4.1.6 Boric acid, solution, $c(H_3BO_3) \approx 0,17$ mol/dm³.

Dissolve 40 g of solid boric acid in 1 dm³ of water, warming if necessary, and 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 cm³ of 96 % (V/V) ethanol.

This indicator may 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 cm³.

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 (see 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 cm³ of the sulfuric acid (4.1.2), or, alternatively, 65 cm³ 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 a further 1 h.

Allow the digestion flask and its contents to cool to room temperature, cautiously add 200 cm³ of water and mix by swirling.

Place the receiving flask containing the absorbing solution in position, connect the distillation unit, then slowly add 150 cm³ 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.

4.4.2.1 Place 75 cm³ of water and, by means of a pipette, 25 cm³ 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 cm³ of distillate have been collected. 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 (normally when the volume in the flask reaches about 300 cm³), titrate the contents with the sodium hydroxide solution (4.1.4), reading the burette to the nearest 0,02 cm³.

4.4.2.2 Place 100 cm³ 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 cm³.

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 results¹⁾

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 the formula

$$\frac{0,14(V_2 - V_1)}{m}$$

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;

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

Express the result 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 the formula

$$\frac{0,14(V_3 - V_4)}{m}$$

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;

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

Express the result 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

- 30 parts by mass of anhydrous potassium sulfate (K_2SO_4);
- 4 parts by mass of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$);
- 1 part by mass of selenium powder, or 2 parts by mass of sodium selenate decahydrate ($Na_2SeO_4 \cdot 10H_2O$).

5.1.2 Sulfuric acid, ρ 1,84 g/cm³.

5.1.3 Sulfuric acid, standard volumetric solution, $c(H_2SO_4) = 0,01$ mol/dm³.

5.1.4 Sodium hydroxide, solution, $c(NaOH) \approx 10$ mol/dm³.

Dissolve 400 g of solid sodium hydroxide in 600 cm³ of water.

5.1.5 Sodium hydroxide, standard volumetric solution, $c(NaOH) = 0,02$ mol/dm³, carbonate-free.

5.1.6 Boric acid, solution, $c(H_3BO_3) \approx 0,17$ mol/dm³.

Dissolve 40 g of solid boric acid in 1 dm³ of water, warming if necessary, and cool the solution to room temperature.

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

5.1.7 Mixed indicator, solution.

Dissolve 0,1 g of methyl red and 0,05 g of methylene blue in 100 cm³ of 96 % (V/V) ethanol.

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

5.2 Apparatus

Ordinary laboratory apparatus and

5.2.1 Semi-micro Kjeldahl digestion apparatus, with digestion flasks of capacity 30 cm³ and 10 cm³ (for an example of a typical apparatus, see figures 1, 2 and 3).

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

5.2.3 Semi-micro burette, of capacity 5 cm³ or 10 cm³, graduated in 0,02 cm³ 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 (see 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 (see 5.2.1). Add about 0,65 g of the catalyst mixture (5.1.1) and 3,0 cm³ 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.

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

Bring the water in the steam generator of the distillation unit to the boil and pass steam through the distillation apparatus (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 cm³ 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 cm³ portions of water and draining the flask thoroughly after each transfer.

5.4.2 Discard any condensate which has 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.

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 cm³ (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 cm³ 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 cm³ 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 cm³. 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 cm³.

5.4.2.2 Place about 10 cm³ 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 cm³.

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 results²⁾

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 the formula

$$\frac{0,028(V_2 - V_1)}{m}$$

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;

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

Express the result 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 the formula

$$\frac{0,028(V_3 - V_4)}{m}$$

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;

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

Express the result to the nearest 0,01 %.

6 Precision

6.1 General

The precision calculations to express repeatability and reproducibility were performed in accordance with ISO/TR 9272. Consult this Technical Report for precision concepts and nomenclature. Annex A of this International Standard gives guidance on the use of repeatability and reproducibility.

6.2 Precision details

6.2.1 An interlaboratory test programme was organized in late 1984 by the Rubber Research Institute of Malaysia. Two separate programmes were conducted, one in March and one in July. Two types of material were sent to each laboratory:

- blended samples of two rubbers "A" and "B";
- unblended (normal) samples of the same two materials "A" and "B".

6.2.2 For both the blended and the unblended samples, a test result was taken as the mean of three separate determinations.

6.2.3 The semi-micro method was used to determine the nitrogen content.

6.2.4 A "type 1" precision was measured in the interlaboratory test programme. The time period for repeatability and reproducibility was on a scale of days. A total of 14 laboratories participated in the programme for blended samples and a total of 13 laboratories in the programme for unblended samples.

6.3 Precision results

The precision results for the blended-sample programme are given in table 1 and the results for the unblended-sample programme in table 2.

7 Test report

The test report shall include the following information:

- a reference to this International Standard and the method used;
- all details necessary for the identification of the material tested;
- the results and the units in which they are expressed;
- any unusual features noted during the determination;
- any operation not included in this International Standard or in the International Standards to which reference is made, and any operation regarded as optional;
- the date of the test.

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

Table 1 — Type 1 precision — Blended-sample testing

Rubber sample	Average nitrogen content % (m/m)	Within-laboratory repeatability		Interlaboratory reproducibility	
		<i>r</i>	(<i>r</i>)	<i>R</i>	(<i>R</i>)
A	0,45	0,053	11,7	0,094	20,9
B	0,53	0,024	4,45	0,161	30,2
Pooled values	0,49	0,042	8,42	0,127	25,6
<i>r</i> = repeatability, in percent by mass (<i>r</i>) = repeatability, in percent (relative) of the average <i>R</i> = reproducibility, in percent by mass (<i>R</i>) = reproducibility, in percent (relative) of the average					

Table 2 — Type 1 precision — Unblended-sample testing

Rubber sample	Average nitrogen content % (m/m)	Within-laboratory repeatability		Interlaboratory reproducibility	
		<i>r</i>	(<i>r</i>)	<i>R</i>	(<i>R</i>)
A	0,36	0,021	5,83	0,189	52,7
B	0,36	0,038	10,82	0,185	51,9
Pooled values	0,36	0,031	8,67	0,187	52,4
See table 1 for symbol definitions.					

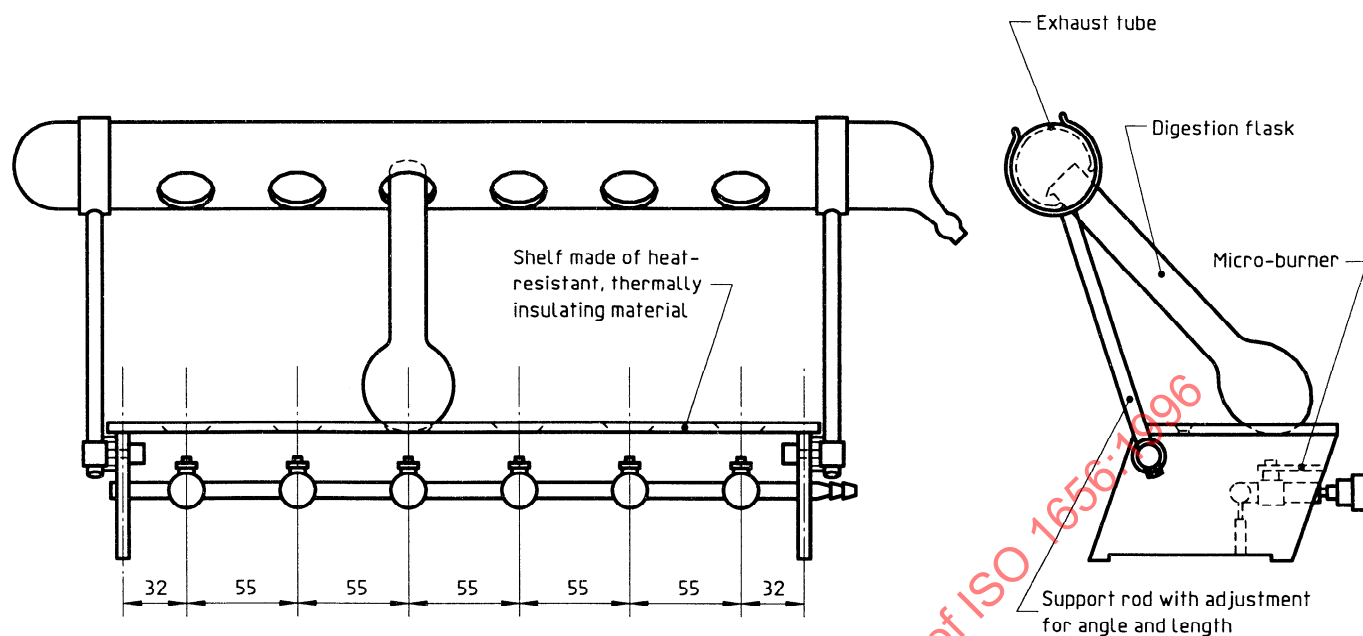
Dimensions in millimetres³⁾

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

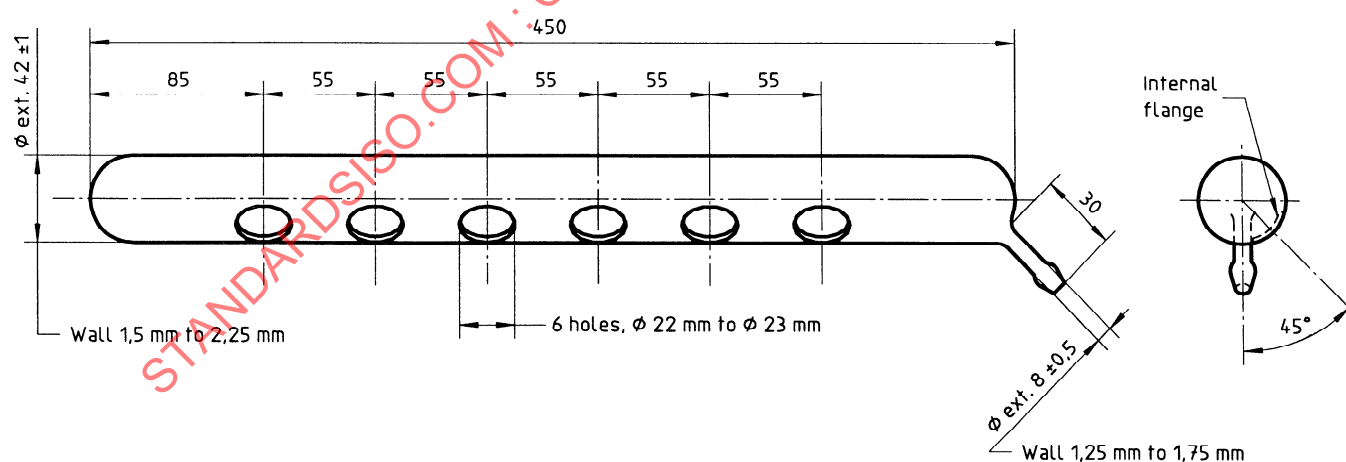
Dimensions in millimetres³⁾

Figure 2 — Exhaust tube for the semi-micro method

3) Where no tolerances are shown, normal working tolerances are allowed.

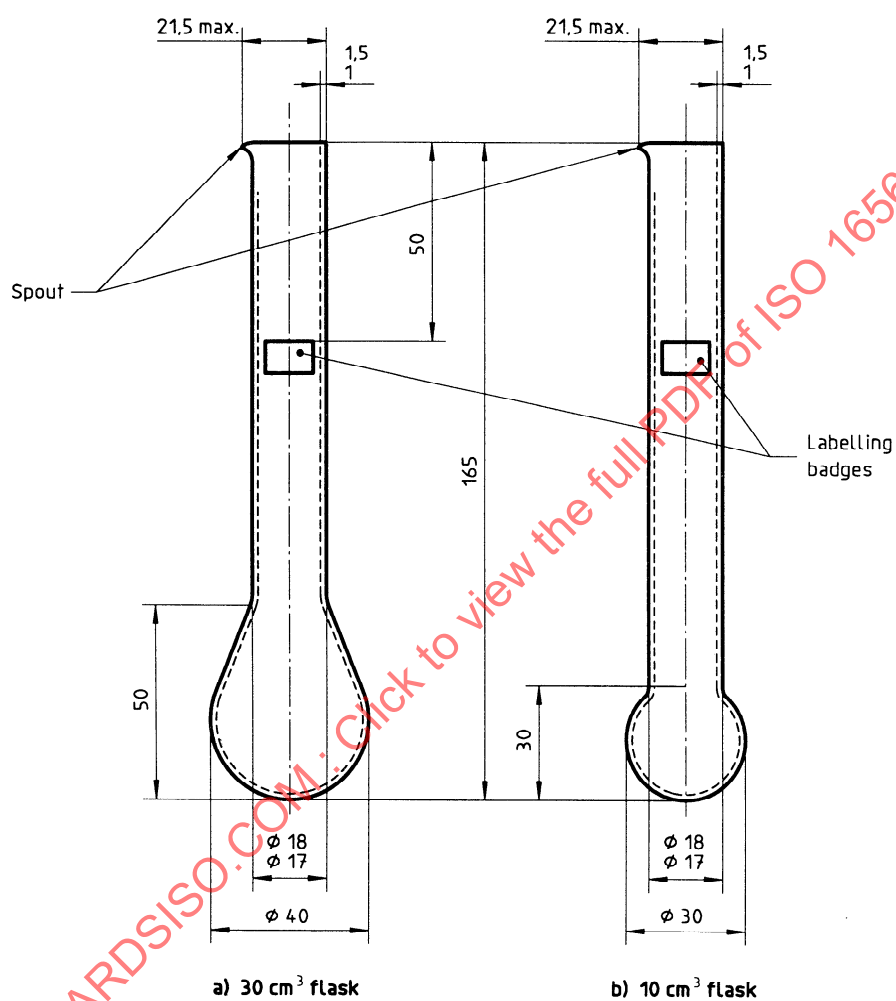
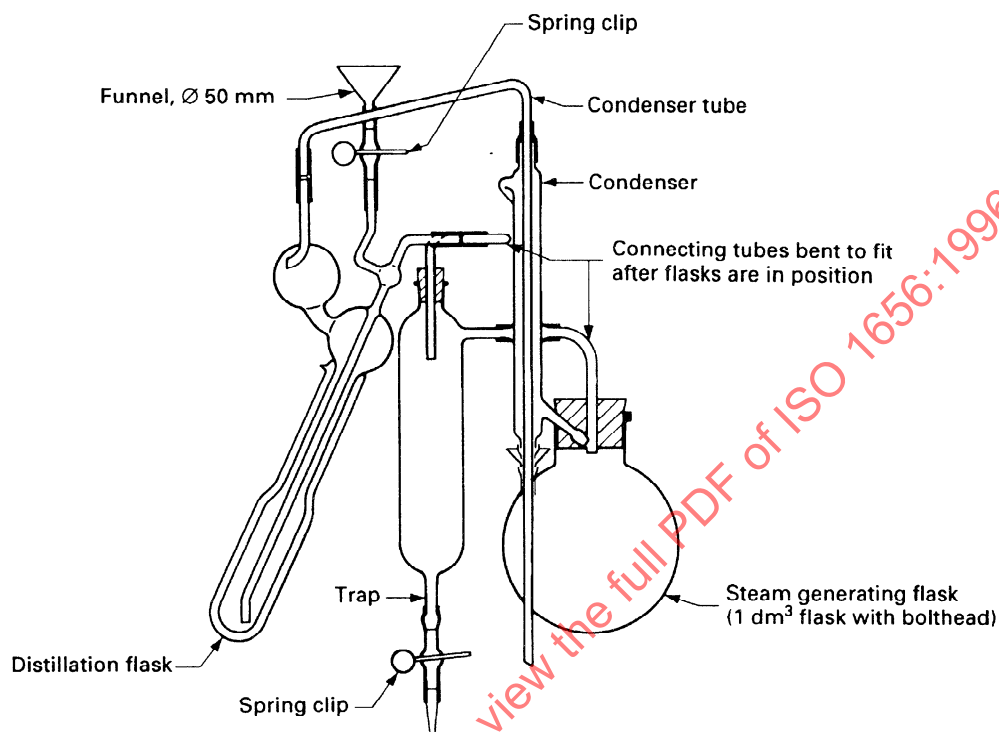
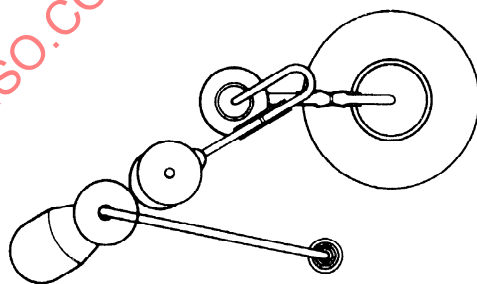
Dimensions in millimetres⁴⁾

Figure 3 — Digestion flasks for the semi-micro method

4) Where no tolerances are shown, normal working tolerances are allowed.



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



Plan

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

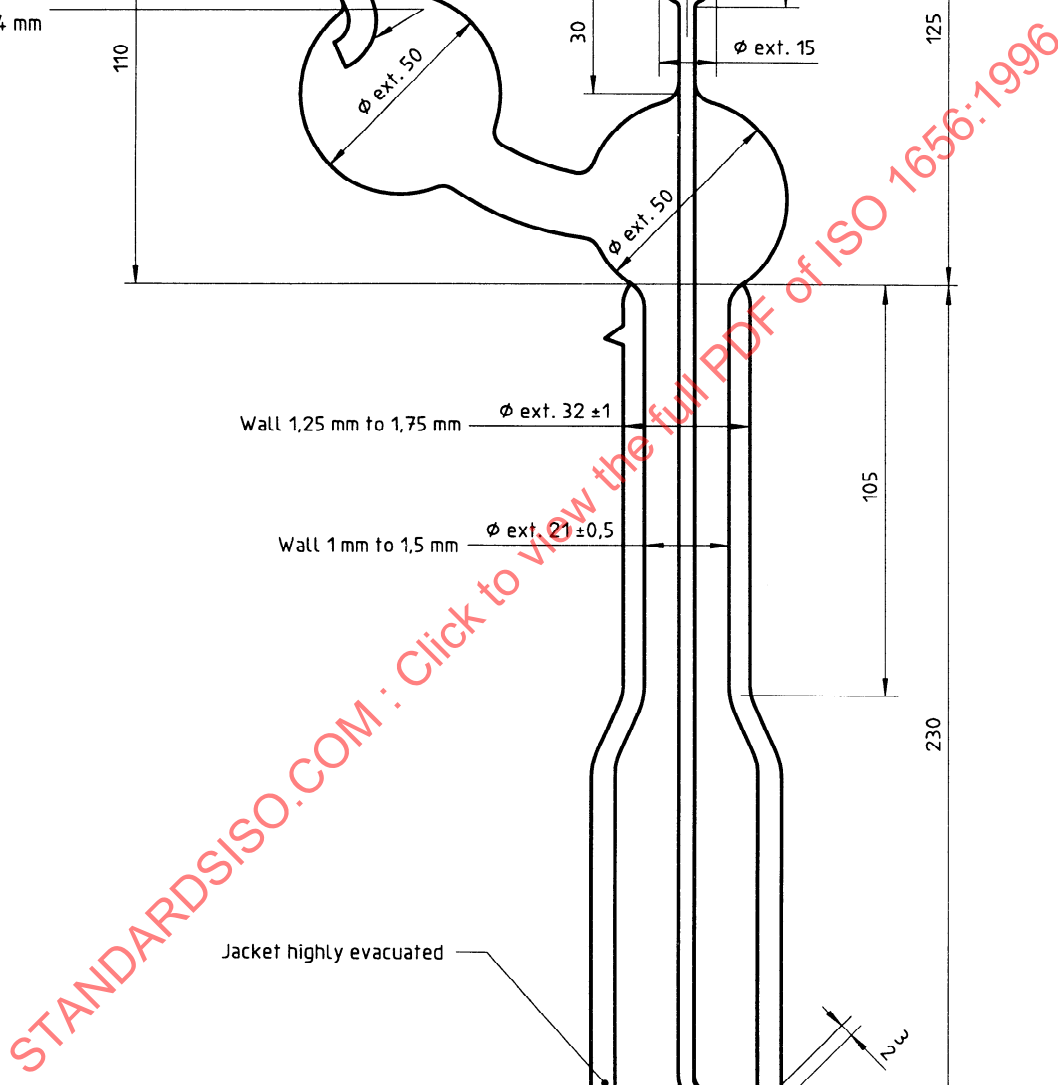


Figure 5 — Distillation flask for the semi-micro method

5) Where no tolerances are shown, normal working tolerances are allowed.