INTERNATIONAL STANDARD ISO 1656

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

Raw natural rubber and natural rubber latex — Determination of nitrogen

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

First edition — 1974-02-01

UDC 678.031/.032 : 543.846

Ref. No. ISO 1656-1974 (E)

Descriptors : elastomers, natural rubber, latex, chemical analysis, determination of content, nitrogen

Price based on 4 pages

FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO Member Bodies). The work of developing International Standards is carried out through ISO Technical Committees. Every Member Body interested in a subject for which a Technical Committee has been set up 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.

Draft International Standards adopted by the Technical Committees are circulated to the Member Bodies for approval before their acceptance as International Standards by the ISO Council.

Prior to 1972, the results of the work of the Technical Committees were published as ISO Recommendations; these documents are now in the process of being transformed into International Standards. As part of this process, Technical Committee ISO/TC 45 has reviewed ISO Recommendation R 1656 and found it suitable for transformation. International Standard ISO 1656 therefore replaces ISO Recommendation R 1656-1971.

ISO Recommendation R 1656 was approved by the Member Bodies of the following countries :

Australia
Austria
Brazil
Canada
Colombia
Czechoslovakia
Egypt, Arab Rep. of
France
Germany
Greece
Hungary
India
Iran
Israel
Italy
Korea, Rep. of
Netherlands
New Zealand
Poland
Spain
Sri Lanka
Sweden
Switzerland
Thailand
Turkey
United Kingdom
U.S.A.
U.S.S.R.

The Member Body of the following country has subsequently approved this Recommendation :

South Africa, Rep. of

No Member Body expressed disapproval of the Recommendation.

No Member Body disapproved the transformation of ISO/R 1656 into an International Standard.

Printed in Switzerland

Raw natural rubber and natural rubber latex – Determination of nitrogen

1 SCOPE AND FIELD OF APPLICATION

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 the 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 REFERENCES

ISO 124, *Rubber latex – Determination of total solids content.* [1]

ISO 1795, *Raw rubber in bales – Sampling.*

ISO 1796, *Raw rubber – Sample preparation.*

3 PRINCIPLE

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

Absorption of the distilled ammonia either

- in standard sulphuric acid followed by titration of the excess acid with a standard base, or
- in boric acid solution followed by titration with standard acid.

4 MACRO METHOD

4.1 Reagents

All reagents shall be of recognized analytical quality and distilled water shall be used where water is specified.

4.1.1 Zinc, granulated.

4.1.2 Catalyst mixture or **catalyst solution**

a) Catalyst mixture. Prepare a finely divided intimate mixture of :

- 30 parts by mass of anhydrous potassium sulphate;
- 4 parts by mass of copper sulphate pentahydrate ($CuSO_4.5H_2O$);
- 1 part by mass of selenium powder, or 2 parts by mass of sodium selenate.

b) Catalyst solution. Dissolve, with heating :

- 110 g of anhydrous potassium sulphate;
- 14,7 g of copper sulphate pentahydrate;
- 3,7 g of selenium, or 7,49 g of sodium selenate;

in 600 ml of sulphuric acid (ρ 1,84 g/ml).

4.1.3 Sulphuric acid, ρ 1,84 g/ml.

4.1.4 Sulphuric acid, 0,1 N solution.

4.1.5 Sodium hydroxide, 0,1 N solution.

---

[1] At present at the stage of draft. (Revision of ISO/R 124.)

4.1.6 **Sodium hydroxide** solution.

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

4.1.7 **Boric acid** solution.

Dissolve 40 g of solid boric acid (H_3BO_3) in water, warming if necessary, and cool the solution to room temperature. Make up the volume to 1 l.

4.1.8 Indicator.

Dissolve 0,1 g of methyl red and 0,05 g of methylene blue in 100 ml of 96 % (*V/V*) ethanol. This indicator may deteriorate during storage and shall therefore be freshly prepared.

4.2 Apparatus

Ordinary laboratory apparatus and

4.2.1 Kjeldahl apparatus, with an 800 ml digestion flask.

4.3 Preparation of test portion

For the determination of nitrogen in raw solid rubber, a test portion from the homogenized piece shall be selected in accordance with ISO 1795 and ISO 1796.

For the determination of nitrogen in latex, a portion 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

Cut about 2 g of the rubber, weighed to the nearest 0,5 mg, into small pieces and place in the 800 ml Kjeldahl flask. Add about 13 g of the catalyst mixture (4.1.2 a)) and 60 ml of sulphuric acid (4.1.3), or, as an alternative, 65 ml of the catalyst solution (4.1.2 b)). Mix the contents of the flask by swirling, and then boil gently until the solution is clear. Continue the boiling for a further 1 h.

When the flask and its contents have cooled to room temperature, cautiously add 200 ml of water and mix by swirling. Then pour 150 ml of sodium hydroxide solution (4.1.6) slowly down the neck of the flask so that the contents do not mix and the acid and alkali layers remain distinct. Carefully introduce one or two pieces of zinc (4.1.1) into the flask and immediately connect it to the distillation unit.

Either of the following procedures may be used for absorbing and titrating the liberated ammonia :

a) Place 75 ml of water and, by pipette, 25 ml of sulphuric acid solution (4.1.4) in the receiving flask of the distillation unit, together with two drops of indicator solution (4.1.8). 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 Kjeldahl flask in place, thoroughly mix the contents by swirling. Immediately commence distillation and continue at a steady rate until 200 ml of distillate have been collected. If the colour of the indicator changes, indicating alkalinity of the absorbing solution, add a further measured quantity of sulphuric acid solution (4.1.4) to restore acid conditions. When the distillation is complete (normally when the volume in the flask reaches about 300 ml) titrate the contents with sodium hydroxide solution (4.1.5).

b) Place 100 ml of boric acid solution (4.1.7) in the receiving flask of the distillation unit with two drops of indicator solution (4.1.8). Carry out the distillation as described in paragraph a) and titrate the distillate with sulphuric acid solution (4.1.4).

In parallel with the determination, carry out a blank test using the same quantities of reagents and under the same conditions.

NOTE — If extra sulphuric acid solution (4.1.4) is added to the absorbing solution in the determination, the same amount shall be added in the blank test.

4.5 Expression of results

4.5.1 When sulphuric acid is used as the absorbing solution as specified in 4.4 a), the nitrogen content of the rubber is given, as a percentage by mass, by the formula :

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

where

V_1 is the volume, in millilitres, of 0,1 N sodium hydroxide solution required to titrate the distillate from the determination;

V_2 is the volume, in millilitres, of 0,1 N sodium hydroxide solution required to titrate the distillate from the blank test;

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

4.5.2 When boric acid is used as the absorbing solution as specified in 4.4 b), the nitrogen content of the rubber is given, as a percentage by mass, by the formula :

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

where

V_3 is the volume, in millilitres, of 0,1 N sulphuric acid solution required to titrate the distillate from the determination;

V_4 is the volume, in millilitres, of 0,1 N sulphuric acid solution required to titrate the distillate from the blank test;

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

5 SEMI-MICRO METHOD

5.1 Reagents

All reagents shall be of recognized micro-analytical quality and distilled water shall be used where water is specified.

5.1.1 Catalyst mixture.

Prepare a finely divided intimate mixture of :

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

5.1.2 Sulphuric acid, ρ 1,84 g/ml.

5.1.3 Sulphuric acid, 0,02 N solution.

5.1.4 Sodium hydroxide, 10 N solution.

5.1.5 Sodium hydroxide, 0,02 N solution, carbonate free.

5.1.6 Boric acid, 0,5 N solution.

5.1.7 Indicator.

Dissolve 0,1 g of methyl red and 0,05 g of methylene blue in 100 ml 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 flasks, capacity 30 ml.

5.2.2 Semi-micro Kjeldahl distillation unit, with condenser tube of silver, boro-silicate glass or tin.

5.2.3 Burette, 5 ml, graduated in 0,02 ml.

5.3 Preparation of test portion

For the determination of nitrogen in raw solid rubber, a test portion from the homogenized piece shall be selected in accordance with ISO 1795 and ISO 1796.

For the determination of nitrogen in latex, a portion 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

Weigh about 0,1 g of the rubber, to the nearest 0,5 mg, and place in a 30 ml Kjeldahl flask (5.2.1). Add about 0,65 g of the catalyst mixture (5.1.1) and 3,0 ml of sulphuric 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 a clear green colour with no yellow tint.

NOTE — Excess boiling, as indicated by a tendency for the digest to solidify on cooling, shall be avoided, 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 apparatus, including the receiving flask, for at least 2 min. The water-jacket of the condenser shall be emptied of water during the steaming-out operation. Meanwhile, cool the Kjeldahl flask to room temperature or below, add 10 ml of water and immediately transfer the contents to the distillation flask at the conclusion of the steaming-out process. Complete the transfer by rinsing with three 3 ml portions of water and draining the flask thoroughly after each transfer to minimize drainage error. Discard any condensate which has collected in the receiver and complete the distillation and titration of ammonia by one of the following procedures :

a) Add, from the semi-micro burette (5.2.3), 5 ml (or appropriately more, depending on the amount of nitrogen anticipated) of sulphuric acid solution (5.1.3) to the steamed-out receiver of the distillation apparatus, together with two drops of indicator solution (5.1.7) and about 5 ml of water. Position the flask 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 ml of 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 to 12 min at such a rate that the final volume of liquid in the receiver is about 70 ml. If the colour of the indicator changes, indicating alkalinity of the absorbing solution, add a further measured quantity of sulphuric acid solution (5.1.3) to restore acid solutions. At the conclusion of the distillation, lower the receiving flask until the tip of the condenser is above the level of the acid, continue the distillation for 1 min longer, and then rinse the tip of the condenser tube with a few millilitres of water, which shall be collected in the distillate. Immediately titrate the contents of the receiver with sodium hydroxide solution (5.1.5).

b) Place about 10 ml of boric acid solution (5.1.6) in the steamed-out receiving flask with two drops of indicator solution (5.1.7). Carry out the distillation as described in paragraph a) and titrate the distillate with sulphuric acid solution (5.1.3).

In parallel with the determination, carry out a blank test using the same quantities of reagents and under the same conditions.

NOTE — If extra sulphuric acid solution (5.1.3) is added to the absorbing solution in the determination, the same amount shall be added to the blank test.

5.5 Expression of results

5.5.1 When sulphuric acid is used as the absorbing solution as specified in 5.4 a), the nitrogen content of the rubber is given, as a percentage by mass, by the formula :

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

where

V_1 is the volume, in millilitres, of 0,02 N sodium hydroxide solution required to titrate the distillate from the determination;

V_2 is the volume, in millilitres, of 0,02 N sodium hydroxide solution required to titrate the distillate from the blank test;

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

5.5.2 When boric acid is used as the absorbing solution as specified in 5.4 b), the nitrogen content of the rubber is given, as a percentage by mass, by the formula :

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

where

V_3 is the volume, in millilitres, of 0,02 N sulphuric acid solution required to titrate the distillate from the determination;

V_4 is the volume, in millilitres, of 0,02 N sulphuric acid solution required to titrate the distillate from the blank test;

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

NOTE — If the solutions are not of the exact normality stated, appropriate correction factors shall be used in the calculations.

6 TEST REPORT

The test report shall include the following information :

a) the reference of the method used;

b) the results and the method of expression used;

c) any unusual features noted during the determination;

d) any operation not included in this International Standard or regarded as optional.