UDC 678.031/.032 : 543.846

Ref. No. : ISO/R 1656-1971 (E)

Transformed

ISO

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION

ISO RECOMMENDATION
R 1656

RAW NATURAL RUBBER AND NATURAL RUBBER LATEX

DETERMINATION OF NITROGEN

1st EDITION

May 1971

For each individual country the only valid standard is the national standard of that country.

Printed in Switzerland

Also issued in French and Russian. Copies to be obtained through the national standards organizations.

BRIEF HISTORY

The ISO Recommendation R 1656, *Raw natural rubber and natural rubber latex – Determination of nitrogen,* was drawn up by Technical Committee ISO/TC 45, *Rubber,* the Secretariat of which is held by the British Standards Institution (BSI).

Work on this question led to the adoption of Draft ISO Recommendation No. 1656, which was circulated to all the ISO Member Bodies for enquiry in July 1968. It was approved, subject to a few modifications of an editorial nature, by the following Member Bodies :

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

No Member Body opposed the approval of the Draft.

This Draft ISO Recommendation was then submitted by correspondence to the ISO Council, which decided to accept it as an ISO RECOMMENDATION.

ISO Recommendation R 1656 May 1971

RAW NATURAL RUBBER AND NATURAL RUBBER LATEX

DETERMINATION OF NITROGEN

1. SCOPE

This ISO Recommendation describes 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, and in the dry solids prepared from natural rubber latex these materials can make a substantial contribution to the total nitrogen content.

2. PRINCIPLE OF METHODS

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.

3. MACRO METHOD

3.1 Reagents

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

3.1.1 *Zinc,* granulated.

3.1.2 *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).

3.1.3 *Sulphuric acid,* ρ 1.84 g/ml.

3.1.4 *Sulphuric acid* solution 0.1 N.

3.1.5 *Sodium hydroxide* solution 0.1 N.

3.1.6 *Sodium hydroxide* solution.

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

3.1.7 *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 litre.

3.1.8 *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 should therefore be freshly prepared.

3.2 Apparatus

Ordinary laboratory apparatus and

3.2.1 *Kjeldahl apparatus,* with an 800 ml digestion flask.

3.3 Preparation of test portion

For the determination of nitrogen in raw solid rubber, a test portion from the homogenized piece should be selected in accordance with ISO Recommendations R 1795, *Sampling of raw rubber in bales,* and R 1796*, *Sample preparation of raw rubber.*

For the determination of nitrogen in latex, a portion of thoroughly mixed latex containing about 2 g of total solids should be dried to constant mass as described in ISO Recommendation R 124, *Determination of total solids of latex.*

3.4 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 (3.1.2 (*a*)) and 60 ml of sulphuric acid (3.1.3), or, as an alternative, 65 ml of the catalyst solution (3.1.2 (*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 hour.

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 (3.1.6) 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 (3.1.1) 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 (3.1.4) in the receiving flask of the distillation unit, together with two drops of indicator solution (3.1.8). 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 (3.1.4) 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 (3.1.5).

(*b*) Place 100 ml of boric acid solution (3.1.7) in the receiving flask of the distillation unit with two drops of indicator solution (3.1.8). Carry out the distillation as described in paragraph (*a*) and titrate the distillate with sulphuric acid solution (3.1.4).

Concurrently 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 (3.1.4) is added to the absorbing solution in the determination, the same amount should be added in the blank test.

---

* At present at the stage of Draft ISO Recommendation.

3.5 Expression of results

(*a*) When sulphuric acid is used as the absorbing solution as described in clause 3.4 (*a*), the nitrogen content of the rubber is calculated from the formula :

$$\text{Nitrogen, \%} = \frac{0.14\,(V_2 - V_1)}{m}$$

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 grammes, of the test portion.

(*b*) When boric acid is used as the absorbing solution as described in clause 3.4 (*b*), the nitrogen content of the rubber is calculated from the formula :

$$\text{Nitrogen, \%} = \frac{0.14\,(V_3 - V_4)}{m}$$

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 grammes, of the test portion.

4. SEMI-MICRO METHOD

4.1 Reagents

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

4.1.1 *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 ($Na_2SeO_4.10H_2O$).

4.1.2 *Sulphuric acid,* ρ 1.84 g/ml.

4.1.3 *Sulphuric acid* solution, 0.02 N.

4.1.4 *Sodium hydroxide* solution, 10 N.

4.1.5 *Sodium hydroxide* solution, 0.02 N, carbonate free.

4.1.6 *Boric acid* solution, 0.5 N.

4.1.7 *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 should therefore be freshly prepared.

4.2 Apparatus

Ordinary laboratory apparatus and

4.2.1 *Semi-micro Kjeldahl digestion flasks,* capacity 30 ml.

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

4.2.3 *Burette,* 5 ml, graduated in 0.02 ml.

4.3 Preparation of test portion

For the determination of nitrogen in raw solid rubber, a test portion from the homogenized piece should be selected in accordance with ISO Recommendations R 1795, *Sampling of raw rubber in bales,* and R 1796*, *Sample preparation of raw rubber.*

For the determination of nitrogen in latex, a portion of thoroughly mixed latex containing about 0.1 g of total solids should be dried to constant mass as described in ISO Recommendation R 124, *Determination of total solids of latex.*

4.4 Procedure

Weigh about 0.1 g of the rubber, to the nearest 0.5 mg and place in a 30 ml Kjeldahl flask (4.2.1). Add about 0.65 g of the catalyst mixture (4.1.1) and 3.0 ml of sulphuric acid (4.1.2) and heat the contents carefully to the boiling point. Continue boiling for a further 30 minutes after the digest has become a clear green colour with no yellow tint.

At the same time commence a blank test using the same quantities of reagents taken from the same containers as those used in testing the sample. Heat the blank and test portion similarly throughout the procedure. Excess boiling, as indicated by a tendency for the digest to solidify on cooling, should 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 minutes. The water jacket of the condenser should 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 (4.2.3), 5 ml (or appropriately more, depending on the amount of nitrogen anticipated) of sulphuric acid solution (4.1.3) to the steamed-out receiver of the distillation apparatus, together with two drops of indicator solution (4.1.7) 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 (4.1.4) to the distillation flask by means of a measuring cylinder, and pass steam from the generator through the distillation flask for 10 to 12 minutes 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 (4.1.3) to restore acid conditions. 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 minute longer, and then rinse the tip of the condenser tube with a few millilitres of water, which should be collected in the distillate. Immediately titrate the contents of the receiver with sodium hydroxide solution (4.1.5).

(*b*) Place about 10 ml of boric acid solution (4.1.6) in the steamed-out receiving flask with two drops of indicator solution (4.1.7). Carry out the distillation as described in paragraph (*a*) and titrate the distillate with sulphuric acid solution (4.1.3).

---

* At present at the stage of Draft ISO Recommendation.