International Standard 5377

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

Starch hydrolysis products — Determination of reducing power and dextrose equivalent — Lane and Eynon constant titre method

Produits d'hydrolyse de l'amidon ou de la fécule — Détermination du pouvoir réducteur et de l'équivalent en dextrose — Méthode Lane et Eynon à titre constant

First edition — 1981-12-15

UDC 664.28 : 664.162.036 : 543.24 **Ref. No. ISO 5377-1981 (E)**

Descriptors : starches, tests, determination, mass losses, dry matter, titration.

Price based on 6 pages

Foreword

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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.

International Standard ISO 5377 was developed by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products)*, and was circulated to the member bodies in November 1980.

It has been approved by the member bodies of the following countries :

Australia	Netherlands
Austria	Romania
Egypt, Arab Rep. of	South Africa, Rep. of
France	USA
Iran	USSR

No member body expressed disapproval of the document.

Starch hydrolysis products — Determination of reducing power and dextrose equivalent — Lane and Eynon constant titre method

1 Scope and field of application

This International Standard specifies a Lane and Eynon constant titre method for the determination of the reducing power and dextrose equivalent of all starch hydrolysis products.

2 References

ISO 385/1, *Laboratory glassware — Burettes — Part 1: General requirements.*[1]

ISO 385/2, *Laboratory glassware — Part 2: Burettes for which no waiting time is specified.*[1]

ISO 648, *Laboratory glassware — One-mark pipettes.*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks.*

ISO 1741, *Dextrose — Determination of loss in mass on drying — Vacuum oven method.*

ISO 1742, *Glucose syrup — Determination of dry matter content — Vacuum oven method.*

ISO 1743, *Glucose syrup and dextrose — Determination of dry matter content — Refractive index method.*

ISO 1773, *Laboratory glassware — Boiling flasks (narrow-necked).*

ISO 5809, *Starch, including derivatives and by-products — Determination of sulphated ash.*[2]

3 Definitions

3.1 reducing power : The content of reducing sugars, expressed as the number of grams of anhydrous D-glucose per 100 g of the sample, when determined by the method specified in this International Standard.

3.2 dextrose equivalent : The content of reducing sugars, expressed as the number of grams of anhydrous D-glucose per 100 g of the dry matter in the sample, when determined by the method specified in this International Standard.

4 Principle

Titration of a prescribed volume of mixed Fehling's solution with a solution of a test portion under specified conditions, using methylene blue as internal indicator.

5 Reagents

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

5.1 Fehling's stock solutions

Prepare the following solutions, using apparatus in accordance with clause 6.

5.1.1 Stock solution A

Copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$)	69,3 g
Water to	1 000,0 ml

5.1.2 Stock solution B

Potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$)	346,0 g
Sodium hydroxide (NaOH)	100,0 g
Water to	1 000,0 ml

Before use, decant the clear solution from any sediment that may form.

5.1.3 Mixed Fehling's solution

Transfer into a dry stock glass bottle, in the following order, 100 ml of solution A (5.1.1) and 100 ml of solution B (5.1.2). Mix well.

NOTE — Do not keep Fehling's solution. Prepare this mixed solution just before use and standardize it as specified in 7.1.

1) At present at the stage of draft. (Revision, in part, of ISO/R 385.)

2) At present at the stage of draft.

5.2 Anhydrous D-glucose, complying with the following requirements :

a) a solution containing 400 g/l shall be free from haze and sediment and shall show no more colour than the water used in its preparation when examined downwards in 50 ml Nessler tubes (6.5) filled to the marks;

b) the sulphated ash content shall be not greater than 0,01 % (m/m) when determined by the method specified in ISO 5809 amended as follows :

1) the mass of the test portion shall be increased to 20 g,

2) only a platinum dish shall be used for incineration,

3) the platinum dish shall be weighed to the nearest 0,1 mg before and after the incineration;

c) the maltose and/or *iso*maltose content shall not exceed 0,1 % (m/m) and no sugar of greater relative molecular mass shall be detected.

5.3 D-glucose, standard reference solution.

5.3.1 Determine the dry matter content of the anhydrous D-glucose by the method specified in ISO 1741.

5.3.2 Weigh, to the nearest 0,1 mg, a mass of the anhydrous D-glucose (5.2) containing 0,600 g of solids, dissolve it in water, transfer the solution quantitively into a 100 ml one-mark volumetric flask (6.4), dilute to the mark with water and mix.

Prepare this solution freshly on each day of use.

5.4 Methylene blue ($C_{16}H_{18}ClN_3S{\cdot}2H_2O$), indicator, 1 g/l aqueous solution.

6 Apparatus

Ordinary laboratory apparatus and in particular

6.1 Narrow-necked boiling flask, of capacity 250 ml, complying with the requirements of ISO 1773.

6.2 Burette, of capacity 25 ml, graduated in subdivisions of 0,05 ml, complying with the requirements of ISO 385/2, class A.

6.3 One-mark pipettes, of capacity 1 ml and 25 ml, complying with the requirements of ISO 648, class A.

6.4 One-mark volumetric flasks, of capacity 100 ml, 500 ml and 1 000 ml, complying with the requirements of ISO 1042, class A.

6.5 Nessler tubes, of capacity 50 ml.

6.6 Heating device, suitable for maintaining boiling as required in 7.1.4 whilst enabling the colour change at the end-point of the titration to be observed without the flask having to be removed from the heating device.

6.7 Stop-watch.

7 Procedure

NOTES

1 A boiling aid (for example glass beads) may be added, if desired, to ensure freedom from super-heating.

2 Shield the burette from the source of heat (6.6) at all times.

7.1 Standardization of the mixed Fehling's solution (5.1.3)

7.1.1 Using a pipette (6.3), place 25,0 ml of the Fehling's solution (5.1.3) in the clean, dry boiling flask (6.1).

7.1.2 Fill the burette (6.2) to the zero mark with the D-glucose standard reference solution (5.3).

7.1.3 Run into the boiling flask from the burette, 18,0 ml of the D-glucose solution (5.3). Swirl the flask to mix the contents.

7.1.4 Place the boiling flask on the heating device (6.6), previously adjusted so that the boiling commences in 120 ± 15 s as timed by the stop-watch (6.7).

Do not adjust the heating device subsequently. This ensures that once boiling has commenced the evolution of steam is brisk and continuous throughout the whole of the titration process, thus preventing to the maximum possible extent the entrance of air to the titration flask with consequent re-oxidation of its contents.

7.1.5 Bring the contents of the flask to the boil and continue boiling for 120 s (timed by the stop-watch). Add 1 ml of the methylene blue solution (5.4) towards the end of this period. After expiry of the 120 s period, commence adding the D-glucose solution to the flask from the burette in 0,5 ml increments until the colour of the methylene blue is discharged, boiling being continued during the whole titration.

Note the total volume of the D-glucose solution (V ml), added up to and including the penultimate 0,5 ml increment.

NOTE — The disappearance of the colour of the methylene blue is best seen by looking at the upper layers and the meniscus of the contents of the titration flask, as these will be relatively free from the precipitated, red copper(I) oxide. The colour disappearance is more easily seen when indirect lighting is used. A white screen behind the titration flask is helpful.

7.1.6 Repeat 7.1.1 and 7.1.2.

7.1.7 From the burette run into the boiling flask a volume of the D-glucose solution equal to (V — 0,3) ml.

7.1.8 Repeat 7.1.4.

7.1.9 Bring the contents of the flask to the boil and continue boiling for 120 s (timed by the stop-watch). Add 1 ml of the methylene blue solution towards the end of this period. After expiry of the 120 s period, commence adding the D-glucose solution to the flask from the burette, initially in 0,2 ml increments and finally drop by drop, until the colour of the methylene blue (5.4) is just discharged, boiling being continued during the whole titration.

Towards the end of this action, add the successive increments of the D-glucose solution at intervals of 10 to 15 s. Complete these additions within 60 s to give a total time of boiling not longer than 180 s.

A third titration with a slightly larger, appropriately adjusted, initial addition of the D-glucose solution may be necessary to achieve this.

7.1.10 Note the volume of the D-glucose solution used up to the end-point of the final titration.

7.1.11 It is essential that the titre is between 19,0 and 21,0 ml of the D-glucose solution. If it is outside these limits, adjust the concentration of the Fehling's solution A (5.1.1) appropriately and repeat the standardization process.

7.1.12 Repeat 7.1.6 to 7.1.10 and calculate the mean of the two titres (V_1 ml).

7.1.13 For the day-to-day standardization of the mixed Fehling's solution, as V_1 is known with accuracy, a single titration only is necessary, using an initial addition of ($V_1 - 0{,}5$) ml of the D-glucose solution.

NOTE — As there is a personal factor involved, it is essential that each operator carries out his own standardization titration and uses his own value of V_1 in the calculation (8.1.1).

7.2 Determination

7.2.1 Preparation of test sample

If the sample is in powder or crystalline form, remove it from its container, break down any lumps, mix in an appropriate manner and place in a suitable airtight container.

If the sample is in massive, solid form, e.g. starch sugar (solid glucose), melt it in a closed container immersed in a hot water bath at 60 to 70 °C, allow to cool to ambient temperature and shake a number of times without removing the closure, in order to mix any condensed moisture on the interior into the sample.

If the sample is in liquid form, mix it by stirring it in its container, after removing any skin that may have formed on its surface.

7.2.2 If the reducing sugar content of the sample is unknown, obtain an approximation to its value by prior trial titrations, in general as specified in 7.1.1 to 7.1.5, but with the following modifications :

a) Add 10,0 ml of the test solution in place of the D-glucose solution added in 7.1.3.

b) After 7.1.4

1) Immediately boiling starts, add 1 ml of the methylene blue solution and commence adding the test solution to the flask from the burette in 1,0 ml increments at intervals of approximately 10 s until the blue colour of the methylene blue is discharged. If the blue colour is discharged before the addition of any 1,0 ml increments of test solution, decrease the concentration of the test solution and repeat the titration.

2) Note the total volume of test solution (V' ml), added up to and including the penultimate increment.

V' shall not be greater than 50 ml. If it is, increase the concentration of the test solution and repeat the titration.

3) The approximate reducing power (see 3.1) (ARP) of the test sample is given by the formula

$$\text{ARP} = \frac{F \times 100 \times 500}{V' \times m_0} = \frac{50\,000 \times F}{V' \times m_0} = \frac{300 \times V_1}{V' \times m_0}$$

where

$F = (0{,}6 \times V_1)/100 = 0{,}006 \times V_1$

m_0 is the mass, in grams, of the test portion in 500 ml of test solution.

The mass, in grams, of the test portion to be taken is

$$\frac{100 \times 3}{\text{ARP}} = \frac{300}{\text{ARP}}$$

7.2.3 Test portion

Weigh, to the nearest 1 mg, a mass of the test sample (m g) containing between 2,85 and 3,15 g of reducing sugars (expressed as anhydrous D-glucose).

7.2.4 Test solution

Dissolve the test portion (7.2.3) in water, transfer the solution quantitively to a 500 ml one-mark volumetric flask (6.4), dilute to the mark with water and mix.

7.2.5 Titration

7.2.5.1 Repeat 7.1.1 to 7.1.9 using the test solution (7.2.4) in place of the D-glucose solution (5.3).

7.2.5.2 Note the volume (V_2) of the test solution (7.2.4) used up to the end-point of the final titration.

7.2.5.3 It is essential that V_2 is between 19,0 and 21,0 ml of the test solution (7.2.4). If V_2 lies outside these limits, adjust the concentration of the test solution appropriately and repeat 7.2.5.1 and 7.2.5.2.

7.2.5.4 Carry out two determinations on the same test sample.

7.3 Dry matter content

Determine the dry matter content [DMC % (m/m)] of the test sample as follows :

a) For dried glucose syrup, by the method specified in ISO 1742.

b) For dextrose (anhydrous and monohydrate), by the method specified in ISO 1741.

c) For glucose syrup, by the method specified in ISO 1743.

8 Expression of results

8.1 Method of calculation and formulae

8.1.1 Reducing power (Lane and Eynon) (see 3.1)

$$\text{RP} = \frac{0{,}600 \times V_1}{100} \times \frac{500}{V_2} \times \frac{100}{m}$$

$$= \frac{300 \times V_1}{V_2 \times m}$$

8.1.2 Dextrose equivalent (Lane and Eynon) (see 3.2)

$$\text{DE} = \frac{\text{RP} \times 100}{\text{DMC}}$$

where

V_1 is the volume, in millilitres, of the D-glucose solution (5.3) used in the standardization of the mixed Fehling's solution (7.1);

V_2 is the volume, in millilitres, of the test solution (7.2.4) used in the determination (7.2.5);

m is the mass, in grams, of the test portion (7.2.3) used to make 500 ml of the test solution (7.2.4);

DMC is the dry matter content of the test sample, expressed as a percentage by mass (see 7.3).

8.1.3 Report the result as the arithmetic mean of the two determinations, provided that the requirement concerning repeatability (see 8.2) is satisfied.

8.2 Repeatability

The results of two determinations carried out in rapid succession by the same analyst shall not differ by more than 0,75 % of their arithmetic mean.

8.3 Reproducibility

The results reported by two different laboratories on the same test sample shall not differ by more than 1,5 % of their arithmetic mean.

9 Test report

The test report shall include all details required for complete identification of the sample, the date of test, the number of this International Standard, the method used, the results obtained and any operating conditions not described in this International Standard (or regarded as optional) as well as any circumstances that might have influenced the results.