INTERNATIONAL STANDARD

**ISO 11216**

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# Modified starch — Determination of content of carboxymethyl groups in carboxymethyl starch

*Amidons et fécules modifiés — Détermination des groupes carboxyméthyles dans l'amidon carboxyméthylé*

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established 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. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 11216 was prepared by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products)*.

Annexes A and B of this International Standard are for information only.





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# Modified starch — Determination of content of carboxymethyl groups in carboxymethyl starch

## 1 Scope

This International Standard specifies a method for the determination of the content of carboxymethyl groups in carboxymethyl starch. The method is suitable for determining carboxymethyl group contents between 1,6 % (*m*/*m*) and 10,0 % (*m*/*m*).

## 2 Normative references



The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of the publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on the 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 166:1996, *Starch — Determination of moisture content — Oven-drying method.*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

## 3 Definition

For the purposes of this International Standard, the following definition applies.

**3.1**
**carboxymethyl group content**
mass of carboxymethyl groups in the acid form divided by the mass of the test portion of carboxymethyl starch in the acid form

NOTE The carboxymethyl group content is expressed as a percentage by mass.

## 4 Principle

The carboxymethyl groups are converted into the acid form by acidifying a solution or a suspension of the starch with hydrochloric acid. After the starch is precipitated with methanol, it is allowed to settle before being filtered off on a sintered glass crucible. The excess acid is completely removed by washing with methanol. The starch is dried and a weighed portion is treated with a measured excess of sodium hydroxide solution. The sodium hydroxide not used by the sample is back-titrated with hydrochloric acid.

## 5 Reagents and materials

Use only reagents of recognized analytical grade.

**5.1 Water,** complying with at least grade 3 in accordance with ISO 3696. The water shall be free from carbon dioxide.

**5.2 Methanol,** 100 %.

**5.3 Hydrochloric acid,** $c$(HCl) = 4 mol/l.

**5.4 Sodium hydroxide solution,** $c$(NaOH) = 0,1 mol/l, free from carbon dioxide.

**5.5 Phenolphthalein solution,** in ethanol 90 % ($V/V$), $\rho$(phenolphthalein) = 10 g/l.

**5.6 Dilute hydrochloric acid,** $c$(HCl) = 0,1 mol/l.

**5.7 Sodium chloride**.

## 6 Apparatus

Usual laboratory apparatus and**,** in particular, the following.

**6.1 Beakers,** of capacity 150 ml.

**6.2 pH meter**.



**6.3 Mechanical stirrer,** capable of high shear, and being explosion proof.

**6.4 Beakers**, of capacity 500 ml.



**6.5 Crucibles,** sintered glass, 40 ml volume, porosity P40 (pore size index 16 µm to 40 µm).

**6.6 Drying oven,** capable of being maintained at a temperature of 40 °C ± 2 °C, provided with air ventilation and being explosion proof.

**6.7 Pestle and mortar**.

**6.8 Sieve,** 800 µm.

**6.9 Blade mill**.

**6.10 Magnetic stirrer**.

## 7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard.

## 8 Preparation of test sample

Sieve the laboratory sample through the 800 µm sieve (6.8). If the material does not pass through the sieve, then grind the sample with a blade mill (6.9) until it passes completely through the 800 µm sieve. Homogenize the sample.

## 9 Procedure

**WARNING — Methanol is a key material in this analysis and is used in quite large quantities. Take all necessary safety precautions because of the toxicity and flammability of methanol. Carry out all work in an explosion-proof fume cupboard. All mechanical and electrical equipment used shall be explosion proof. In addition, disposal of waste methanol shall be in accordance with legal requirements.**

### 9.1 Test portion

Weigh, to the nearest 1 mg, about 3 g of the prepared test sample into a 150 ml beaker (6.1).

### 9.2 Conversion of the carboxymethyl salts

Wet the test portion with 3 ml of methanol (5.2) and stir with a spatula until homogeneous. Add 75 ml of water (5.1) and stir until fully dispersed.

NOTE For highly viscous starch, 6 ml of methanol and 100 ml of water can be used to give a solution which can be easily stirred.

Acidify this solution with hydrochloric acid (5.3) to pH = 1 using the pH meter (6.2). Stir for 30 min with the stirrer (6.3).

### 9.3 Precipitation of the acid form

Add 300 ml of methanol (5.2) into a 500 ml beaker (6.4). Pour the dissolved test portion solution dropwise into the methanol, while stirring vigorously.



NOTE If the test portion is dispersed in 100 ml of water (see note in 9.2), then 400 ml of methanol has to be used to precipitate the starch.

When addition is complete, continue stirring for one further minute. Cover the beaker and allow to stand for 2 h.



### 9.4 Recovery of the precipitated acid form

Decant the clear liquid from the beaker, collecting it in an appropriate vessel. Filter the residue on a sintered glass crucible (6.5) under vacuum.

When the precipitate is just dry, add 25 ml of methanol (5.2). Stir, and re-apply suction.

Repeat this step until the pH of the filtrate is greater than 3,5. Give one final wash with methanol.

Transfer the precipitate from the crucible to a watch glass and dry for a few hours in the oven (6.6) set at 40 °C.

### 9.5 Test portion for titration

Grind the dry precipitate in a pestle and mortar (6.7).

Weigh, to the nearest 0,1 mg, about 1,5 g into a 150 ml beaker (6.1).

NOTE The mass taken should contain between 0,5 mmol and 1,5 mmol of carboxymethyl groups.

### 9.6 Titration

Moisten the sample with 2 ml of methanol (5.2) and add 75 ml of water (5.1) to dissolve. Warm the contents of the beaker in a boiling water bath to 90 °C. Cool to ambient temperature before continuing.

Add 25,00 ml of sodium hydroxide solution (5.4) to the solution. Cover the beaker with foil and stir for 1 h on a magnetic stirrer (6.10).

Introduce 2 or 3 drops of phenolphthalein solution (5.5) and titrate to just colourless with dilute hydrochloric acid (5.6).

NOTE 1 If the titration is performed potentiometrically, this should be performed in a closed vessel, with pH = 9,0 as endpoint.

NOTE 2 If a very viscous solution is obtained for titration, up to 50 mg of sodium chloride (5.7) can be added to reduce the viscosity.

### 9.7 Blank titration

Add 25,00 ml of sodium hydroxide solution (5.4) to a 150 ml beaker (6.1). Add 2 ml of methanol (5.2) and 75 ml of water (5.1).

Titrate with hydrochloric acid (5.6) as described in 9.6.

### 9.8 Moisture content

Using a test portion of about 1 g, determine the moisture content of the test portion for titration (9.5) in accordance with ISO 1666.

## 10 Calculation

**10.1** Calculate the carboxymethyl group content of the dry test sample by the equation :

$$w_c = \frac{c \times M_c \times (V_b - V_s) \times 100\,\%}{m} \times \frac{100\,\%}{100\,\% - w_m}$$



where



$w_c$ is the carboxymethyl group content, in percentage by mass, of the acid-washed and dry test sample;

$c$ is the concentration, in moles per litre, of dilute hydrochloric acid (5.6) used for the titration;

$M_c$ is the molar mass, in grams per mole, of the carboxymethyl function in the acid form as reacted to the starch ($M_c$ = 58 g/mol);

$V_b$ is the volume, in millilitres, of dilute hydrochloric acid (5.6) used for the blank titration;

$V_s$ is the volume, in millilitres, of dilute hydrochloric acid (5.6) used for the sample titration;

$m$ is the mass, in milligrams, of the test portion for titration (9.5);

$w_m$ is the moisture content, in percentage by mass, of the test sample for titration (9.5) determined in 9.8.

Round the result to the nearest 0,01 % (*m*/*m*).

**10.2** The result may be calculated as the degree of carboxymethyl substitution, which is defined as the ratio of moles of carboxymethyl per mole anhydroglucose.

The degree of carboxymethyl substitution can be calculated in the dry test sample by the equation:

$$x_c = \frac{w_c \times M_a}{(100\,\% - w_c) \times M_c}$$

where

$x_c$ is the degree of carboxymethyl substitution, in mole fraction, in the dry test sample;

$w_c$ is the carboxymethyl group content, in percentage by mass, of the dry test sample;

$M_a$ is the molar mass, in grams per mole, of anhydroglucose ($M_a$ =162 g/mol);

$M_c$ is the molar mass, in grams per mole, of the carboxymethyl function in the acid form as reacted to the starch ($M_c$ = 58 g/mol).

In this case, the result should be rounded to the nearest 0,001 unit.

## 11 Precision

### 11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

### 11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will not in more than 5 % of cases:

— exceed 7,9 % of the mean of the two test results at a level of 1,6 % (*m*/*m*);

— exceed 3,8 % of the mean of the two test results at a level between 5,9 % (*m*/*m*) and 7,5 % (*m*/*m*).



### 11.3 Reproducibility



The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will not in more than 5 % of cases:

— exceed 23,8 % of the mean of the two test results at a level of 1,6 % (*m*/*m*);

— exceed 15 % of the mean of the two test results at a level of 5,9 % (*m*/*m*) to 7,5 % (*m*/*m*).

## 12 Test report

The test report shall specify:

— all information necessary for the complete identification of the sample;

— the sampling method used, if known;

— the test method used;

— the test result obtained;

— if the repeatability has been checked, the final quoted result obtained ;

— all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents that occurred when performing the method which may have influenced the test result(s).

# Annex A
(informative)

# Results of an interlaboratory trial

An international collaborative test involving 14 laboratories was carried out on four different samples of carboxymethyl potato starches.

The results obtained were subjected to statistical analysis in accordance with ISO 5725 [1] to give the precision data shown in table A.1.

**Table A.1**

| Parameter | Sample[1)] | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Number of laboratories retained after eliminating outliers | 13 | 12 | 13 | 12 |
| Number of outliers (laboratories) | 1 | 2 | 1 | 2 |
| Number of accepted results | 26 | 24 | 26 | 24 |
| Mean carboxymethyl group content % (*m*/*m*) | 7,46 | 1,62 | 5,96 | 5,94 |
| Repeatability standard deviation ($s_r$), % (*m*/*m*) | 0,102 | 0,045 | 0,064 | 0,042 |
| Repeatability relative standard deviation, % | 1,36 | 2,78 | 1,08 | 0,71 |
| Repeatability limit ($r$) [$r = 2{,}8 \times s_r$], % (*m*/*m*) | 0,287 | 0,128 | 0,182 | 0,119 |
| Reproducibility standard deviation ($s_R$), % (*m*/*m*) | 0,381 | 0,136 | 0,315 | 0,271 |
| Reproducibility relative standard deviation, % | 5,11 | 8,37 | 5,29 | 4,56 |
| Reproducibility limit ($R$) [$R = 2{,}8 \times s_R$], % (*m*/*m*) | 1,078 | 0,385 | 0,891 | 0,767 |

[1)] All samples were carboxymethyl potato starches.

# Annex B
(informative)

# Bibliography

[1] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn) was used to obtain the precision data.