RESOLUTION OIV/OENO 366/2009

MONOGRAPH ON CARBOXYMETHYLCELLULOSE (CELLULOSE GUM)

THE GENERAL ASSEMBLY

IN VIEW of article 2, paragraph 2 iv of the Agreement of 3 April 2001, establishing the International Organisation of Vine and Wine,

CONSIDERING resolution 2/2008 adopted by the OIV in 2008 concerning the addition of carboxymethylcellulose to white wines and sparkling wines in order to enhance their tartaric stabilisation following a proposal made by the "Specifications of oenological products" experts group

HAS HEREBY DECIDED to add the following monograph to the international Oenological Codex:

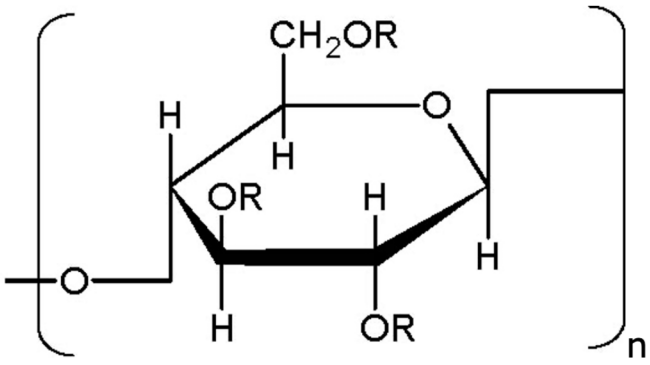
CARBOXYMETHYLCELLULOSE

(cellulose gum)

(CMC)

INS no. 466

CAS [9004-32-4]



where R = H or CH_2COONa

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1. SUBJECT, ORIGIN AND SCOPE

Carboxymethylcellulose (cellulose gum) for oenological use is prepared exclusively from wood by treatment with alkali and monochloroacetic acid or its sodium salt. Carboxymethylcellulose inhibits tartaric precipitation through a "protective colloid" effect. A limited dose is used.

2. SYNONYMS

Cellulose gum, CMC, Sodium CMC, Sodium salt of a carboxymethyl ether of cellulose, NaCMC

3. LABELLING

Labelling must mention that the carboxymethylcellulose is for use in food, as well as safety and preservation conditions.

4. CHARACTERISTICS

4.1. Description

Granular or fibrous powder, blank or slightly yellowish or greyish, slightly hygroscopic, odourless and tasteless. This may be proposed in the form of a concentrate for solution in wine prior to use. Solutions must contain at least 3,5 % carboxymethylcellulose.

4.2. Chemical formula

The polymers contain anhydroglucose units substituted with the following general formula: [$C_6H_7O_2$ OH)x(OCH_2COONa)y]n where

N is the degree of polymerisation

x = from 1.50 to 2.80

y = from 0.2 to 1.50

x + y = 3.0

(y = degree of substitution)

Note: Only the carboxymethylcellulose possessing a degree of substitution between 0.6 and 1.0 are completely soluble.

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4.3. Degree of substitution

Evaluate the degree of substitution using the method described below. The degree of substitution must lie between 0.60 and 0.95.

4.4. Molecular weight

Ranges from 17,000 to 300,000 (degree of polymerisation from 80 to 1,500). The molecular weight can be evaluated through measurement of viscosity.

The viscosity of a 1 % solution must lie between 10 and 15 mPa·s-1, or between 20 and 45 mPa·s-1 for a 2 % solution, or between 200 and 500 mPa·s-1 for a 4 % solution.

4.5. Composition

Measure the carboxymethylcellulose composition using the method described below. The carboxymethylcellulose content must be at least 99.5 % of the anhydrous substance.

5. TESTS

5.1. Solubility

Forms viscous colloidal solution with water. Insoluble in ethanol.

5.2. Foam test

Vigorously shake a 0.1 % solution of the sample. No layer of foam appears (this test distinguishes sodium carboxymethylcellulose from other cellulose ethers and from alginates and natural gums).

5.3. Precipitate Formation

To 5 mL of an 0.5% solution of the sample add 5 mL of a 5 % solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permists the distinction of sodium carboxymethyl cellulose ethers from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum)

5.4. Colour reaction

Add 0.5 g of powdered carboxymethylcellulose sodium to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. To 1 mL of the solution, diluted with an equal volume of water, in a small

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test tube, add 5 drops of 1-naphthol. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface.

5.5. Moisture - Loss on drying

Measure the loss on drying using the method described below. Not more than 12 % after drying.

5.6. pH of a 1 % solution

No less than 6 and no more than 8.5 pH units.

5.7. Arsenic

Quantifythe arsenic using the method described in chapter II. The arsenic content must be lower than 3 mg/kg

5.8. Lead

Quantify the lead using the method described in chapter II. The lead content must be lower than 2 mg/kg

5.9. Mercury

Quantify the mercury using the method described in chapter II. The mercury content must be lower than 1 mg/kg

5.10. Cadmium

Quantify the cadmium using the method described in chapter II. The cadmium content must be lower than 1 mg/kg

5.11. Free Glycolate

Quantify the glycolate using the method described below. The carboxymethylcellulose should not contain more than 0.4 % (calculated in sodium glycolate percentage of the anhydrous substance).

5.12. Sodium

Quantify the sodium using the method described in chapter II. The sodium content must be lower than 12.4 % of the anhydrous substance

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5.13. Sodium chloride

Quantify the sodium chloride using the method described below. The carboxymethylcellulose must not contain more than 0.5 % of the anhydrous substance.

5.14. Loss on drying

5.14.1. Objective

This test determines the volatile part of carboxymethylcellulose. The result of this test is used to calculate the total solids of the sample and by extension, all the volatile substances at the test temperature are regarded as moisture.

5.14.2. Interest and use

The measurement of water content (by taking account of the purity) is used to measure the quantity of carboxymethylcellulose in commercial products.

5.14.3. Equipment

5.14.3.1. Drying oven at 105 °C ± 3 °C;

5.14.3.2. Weighing bottle 50 mm in internal diameter and 30 mm in height or equivalent;

5.14.3.3. Precision balance

5.14.4. Test

5.14.4.1. Weigh between 3 and 5 g of sample to ± 1 mg, in a weighing bottle which has already been tared.

5.14.4.2. Place the weighing bottle without its lid in the drying oven for four hours. Let cool in a desiccator, replace the lid and weigh.

5.14.4.3. Continue the process until constant weight

5.14.5. Calculation

5.14.5.1. Calculate the percentage of the water content M according to the formula:

$M = (A/B) \times 100$ where

A = loss of weight by drying (in g); B = initial mass of sample.

5.15. Sodium Glycolate

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5.15.1. Objective

This test covers the determination of sodium glycolate contained in the purified carboxymethylcellose containing not more than 2 % sodium glycolate.

5.15.2. Summary of the test method

Carboxymethyl cellulose dissolved in acetic acid (50 %), precipitated with acetone in the presence of sodium chloride and the insoluble is eliminated by filtration. The filtrate containing the glycolate sodium (in the form of glycolic acid) is treated to remove the acetone and reacts with 2,7-dihydroxynaphthalene. The resulting colour is measured at 540 nm with a calibrated spectrophotometer using solutions of known concentrations.

5.15.3. Interest and use

This test method (along with moisture and sodium chloride) is must been used when measuring the quantity of polymer in the substance. It must be used to check the purity of carboxymethylcellulose required by public health regulations.

5.15.4. Equipment

5.15.4.1. Spectrophotometer capable of carrying out analysis at 540 nm;

5.15.4.2. Spectrophotometer cells, 1 cm of optical path

5.15.4.3. Aluminium paper in squares approximately 50 × 50 mm;

5.15.4.4. Precision balance

5.15.5. Reagents

5.15.5.1. Acetic acid, glacial (purity ≥ 99 %);

5.15.5.2. Acetone (purity ≥ 99 %);

5.15.5.3. 2,7-dihydroxynaphtalene solution (0.100 g/L): Dissolve 100 mg ± 1 mg of 2,7-dihydroxynaphthalene (naphthalenediol) in 1 L of sulphuric acid. Before using, allow the solution to stand until the initial yellow colo disappears. If the solution is dark, eliminate it and prepare a new one with a different supply of sulphuric acid. This solution remains stable for one month when stored in a dark bottle;

5.15.5.4. Standard glycolic acid solution at 1 mg/mL: dry several grams of glycolic acid in a desiccator for at least sixteen hours at room temperature. Weigh 100 mg ± 1 mg, pour into a 100 mL graduated flask, dissolve with water, adjust with water to the filling mark. Do not keep solution longer than 30 days;

5.15.5.5. Sodium chloride (NaCl, purity ≥ 99 %);

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5.15.5.6. Sulphuric acid concentrate (H_2SO_4 purity ≥ 98 %, □≥ 1.84).

5.15.6. Preparation of the calibration curve

5.15.6.1. In a series of five graduated 100 mL volumetric flasks, pour 0, 1, 2, 3 and 4 mL of the glycolic acid reference solution (to 1 mg / mL). Into each flask, add 5 mL of water, then 5 mL of glacial acetic acid, make up with acetone to the filling mark and mix. These flasks contain respectively, 0, 1, 2, 3 and 4 mg of glycolic acid.

5.15.6.2. Pipet 2 mL of each of these solutions and transfer them into five 25 mL graduated flasks. Evaporate the acetone by heating the open graduated flasks, laid out vertically, in a water bath for exactly 20 min. Remove from the water bath and let cool at room temperature.

5.15.6.3. Add 5 mL of 0.100 g/L 2,7-dihydroxynaphtalene solution, mix thoroughly, then add an additional 15 mL of 2,7-dihydroxynaphtalene solution and mix. Cover the mouth of the flasks with a small piece of aluminium foil, place the flasks upright in the water bath for 20 min. Remove from the water bath, let cool at room temperature and add sulphuric acid to the filling mark.

5.15.6.4. Measure the absorbance of each sample at 540 nm against the blank using 1 cm optical depth cells. Plot the absorbance curve according to the corresponding quantity of glycolic acid (in mg) in each flask.

5.15.7. Test method

5.15.7.1. Weigh 0.500 g ± 0.001 g of sample and transfer into a 100 mL beaker. Moisten the sample entirely with 5 mL of acetic acid, followed by 5 mL of water, stir with a glass rod until dissolution is complete (usually requires approximately 15 minutes). Slowly add 50 mL of acetone while stirring, then approximately 1 g of sodium sulphate. Continue to stir for several minutes to ensure complete completely precipitation the carboxymethylcellulose.

5.15.7.2. Filter using a paper filter previously soaked with small amount of acetone, and collect the filtrate in a 100 mL graduated flask. Use 30 mL of acetone to facilitate transfer of solid matter and to wash the filter cake. Make up to the filling mark with acetone and mix.

5.15.7.3. In another 100 mL graduated flask, prepare a blank with 5 mL of water, 5 mL of glacial acetic acid, then make up to the filling mark with acetone and mix.

5.15.7.4. Pipet 2 mL of the sample solution and 2 mL of the blank solution and pour them into two 25 mL graduated flasks. Evaporate the acetone as before (5.15.6.2).

5.15.7.5. Measure the absorbance of the sample and infer the quantity of glycolic acid (in mg) using the calibration curve (5.15.6.4).

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5.15.8. Calculation:

Calculate the content C (in %) of sodium glycolate (free glycolate) contained using the formula:

$$C(\%\ sodium\ glycolate) = \frac{B \times 0.129}{W \times (100 - M)}$$

where

B = glycolic acid (in mg) inferred using the calibration curve;

W = quantity of weighed carboxymethylcellulose (in g);

M = water content of the sample (in %);

0.129 = (ratio of the molecular weight of sodium glycolate compared to the molecular weight of the glycolic acid)/10.

Note: if the test is carried out with pre-dehydrated carboxymethylcellulose, the formula becomes:

$$C(\%\ sodium\ glycolate) = \frac{B \times 0.129}{W}$$

W = quantity of carboxymethylcellulose (dry) weighed (in g).

5.16. Sodium chloride

5.16.1. Objective

This test method determines the sodium chloride content of the purified carboxymethylcellulose (> 98 %).

5.16.2. Summary of the test method

The sodium carboxymethylcellulose is dissolved in water and titrated by potentiometry with a silver nitrate solution. Hydrogen peroxide is added to reduce the viscosity of the solution.

5.16.3. Importance and use

This test method (along with moisture and sodium glycolate content) is used to calculate the degree of substitution of carboxymethylcellulose. It must be used to

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analyse highly purified grades of sodium carboxymethylcellulose (> 98 %).

A. Equipment

5.16.4.1. pH-meter capable of reading voltage (in mV), equipped with a silver electrode and a mercury sulphate reference electrode saturated with potassium sulphate.

5.16.4.2. buret, 10 mL

5.16.4.3. Precision balance.

5.16.4.4. 250 mL Erlenmeyer flask.

5.16.4. Reagents

5.16.5.1. Concentrated hydrogen peroxide (30 % in mV) (H_2O2).

5.16.5.2. Concentrated nitric acid (HNO_3) (□ 1.42).

5.16.5.3 Silver nitrate, standard solution (0.1 N) - Dissolve 17.0 g of silvNO_3itrate (Ag) in 1 L of water. Store in an amber glass bottle. Standardise the solution as follows: Dry the sodium chloride (NaCl) for 2 hrs at 120 °C. Weigh 0.65 g ± 0.0001 g in a 250 mL beaker and add 100 mL of water. Place on a magnetic stirrer, add 10 mL of HNO_3, and immerse the electrodes of the pH-meter. Using a buret, add by 0.25 mL fractions the theoretical quantity of the AgNO_3 solution. After each addition, wait approximately 30 seconds before carrying out readings of the corresponding voltages. When approaching the endpoint, decrease the additions to 0.05 mL. Record the voltage (in millivolts) according to the volume (in mL) of the titration solution, continue titration a few mL beyond the endpoint. Trace the potential values obtained in relation to the corresponding volumes of titrated solution, and determine the potential of the equivalence point according to the singular point of the curve obtained.

Calculate the normality, N, as follows:

$$N = (A \times 1000)/(B \times 58.45)$$

where

A = NaCl used in g,

B = added AgNO_3 solution in mL,

58.45 = molecular mass of the NaCl in g,

5.16.5.4 Sodium chloride (NaCl, purity ≥ 99%).

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5.16.5. Test method

5.16.6.1. Weigh 5 g ± 0.0001 g of sample in a 250 mL beaker. Add 50 mL of water and 5 mL of H_2O_2 (30 %). Place the beaker on a steam bath, stirring occasionally until the solution is fluid. If dissolution does not occur within 20 min, add 5 mL of H_2O_2 and heat until dissolution is complete.

5.16.6.2. Cool the beaker, add 100 mL of water and 10 mL of HNO3. Place it on the magnetic stirrer and titrate with the 0.1 N $AgNO_3$ solution (5.16.5.3) up to the equivalence point.

5.16.6. Calculation

5.16.7.1. Calculate the sodium chloride content C (in %) as follows:

$$C = (AN \times 584.5)/[G \times (100 - B)]$$

Where:

A = volume of $AgNO_3$ solution added (in mL);

N = Normality of the $AgNO_3$ solution;

G = weight of the sample used (in g),

B = Moisture, given extemporaneously (in %) as per paragraph 5.14 and 584.5 = molecular

mass of NaCl ×10 (in g).

5.17. Degree of substitution

5.17.1. Objective

This method is used to determine the degree of etherification (of substitution) of the carboxymethylcellulose used.

5.17.2. Summary of the test method

Pre-purified carboxymethylcellulose mineralises in the presence of sulphuric acid. The weight of the residual sodium sulphate enables inference of the sodium content and by extension the degree of substitution.

5.17.3. Importance and use

This test method is used to determine the number of substituent groups added to the

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basic cellulose backbone.

5.17.4. Equipment

5.17.4.1. 500 mL Erlenmeyer flask.
5.17.4.2. Precision balance.
5.17.4.3. Sintered glass filter.
5.17.4.4. Filter-flask.
5.17.4.5. Porcelain crucible.
5.17.4.6. Drying oven at 110 °C.
5.17.4.7. Desiccator.
5.17.4.8. Bunsen burner or muffle furnace at 600 °C.

5.17.5. Reagents

5.17.5.1. Methanol or ethanol (purity ≥ 98 %)
5.17.5.2. 0.1 N silver nitrate ($AgNO_3$)
5.17.5.3. Acetone (purity ≥ 99 %)
5.17.5.4. Sulphuric acid (purity ≥ 96 %)
5.17.5.5. Ammonium carbonate (NH_4HCO_3)

5.17.6. Preparation of the sample

(This step is not necessary if the sample is assumed to contain at least 99.5 % of of carboxymethylcellulose)Weigh 5 g of the sample ± 0.1 mg, and transfer into a 500 mL conical flask. Add 350 mL of methanol or ethanol (80 % volume). Stir the suspension for 30 min. Decant trough a tared glass filtering crucible under gentle suction. At the end of filtration, avoid drawing in air through the crucible. Repeat the treatment until the 0.1 N silver nitrate test for the chloride ions is negative for the crucible. Normally, three washings suffisant. Transfer the carboxymethylcellulose into the same crucible. Eliminate the traces of alcohol by rinsing with acetone. Let the acetone evaporate into the air (under a hood) then in a drying oven at 110 °C until constant weight. Weigh for the first time after two hours. Cool the crucible each time in a desiccator and during the weighing, pay attention to the fact that sodium carboxymethyl cellulose is slightly hygroscopic.

5.17.7. Test method

In a porcelain crucible tared beforehand, weigh 2 g ± 0.1 mg of dried substance following the preparation above. Char with the Bunsen burner, first carefully with a

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small flame and then for 10 min with a large flame. Cool, then pour 3 to 5 mL of concentrated sulphuric acid onto the residue. Heat carefully with the fuming is finished. After cooling, add about 1 g of ammonium carbonate by pouring the powder onto the entire contents of the crucible. Reheat, initially with a small flame until no more smoke is released then at deep red for 10 min. Repeat the sulphuric acid and ammonium carbonate washing if the residual sodium sulphate still contains carbon. Let the crucible cool in a desiccator and weigh. In place of adding the ammonium carbonate and heating by flame, the crucible can be placed in an over for one hour at approximately 600 °C.

5.17.8. Calculate

Calculate the sodium content of the sample extracted from alcohol by the formula:

$$\%sodium = \frac{a \times 32.38}{b}$$

a = weight of residual sodium sulphate

b = weight of the sample extracted from dry alcohol

5.17.9. Calculte

Calculate the degree of substitution using the formula:

$$Degré\ de\ substitution = \frac{162 \times \%\ sodium}{2300 - (80 \times \%sodium)}$$

5.18. Composition in carboxymethyl cellulose

Calculate the percentage of sodium carboxymethyl cellulose in the sample by deducting 100 % of the sum of percentages of sodium and sodium glycolate (free glycolate), determined separately by the procedures above.

Carboxymethyl cellulose content (in %) = 100 – (% NaCl + % sodium glycolate)

5.19. Measurement of viscosity

5.19.1. Objective

5.19.1.1. This test method determines the viscosity of aqueous carboxymethylcellulose

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solutions within ranging from 10 to 10 000 mPa/s at 25 °C.

5.19.1.2. The concentration to be used for the test must be such that determination of the solution viscosity will be possible within the limits of the test.

5.19.1.3. The results of the carboxymethylcellulose viscosity measurement by the present test method are not necessarily identical to the results obtained with other types of instruments used for the measurement of viscosity.

5.19.1.4. The determinations are calculated on a dry weight, which requires knowledge of the water content of carboxymethylcellulose (see §5.14).

5.19.1.5. The recommended Brookfield spindles and the speeds are shown in table 1, but they can be adapted for greater convenience.

TABLE 1: Spindles and speeds required by the viscometer

Domaine de viscosité, (en mPa/s)	mobile n°	Vitesse (en tr/min)	Echelle	Facteur
10 to 100	1	60	100	1
100 to 200	1	30	100	2
200 to 1000	2	30	100	10
1000 to 4000	3	30	100	40

5.19.2. Interest and use

This test method is used to estimate the molecular weight of carboxymethylcellulose

5.19.3. Equipment

5.19.3.1. Brookfield viscometer.

5.19.3.2. Glass container, approximately 64 mm (2 ½ inches) in diameter and 152 mm (6 inches) tall, straight edged, 40 g capacity (12 oz).

5.19.3.3. Precision balance

5.19.3.4. Mechanical stirrers with a stainless steel blade fastened to a variable speed motor capable of functioning at 900 ± 100 r/min under different load conditions.

5.19.3.5. Water bath, at 25 °C ± 0.5 °C.

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5.19.3.6. Precision thermometer capable of reading temperatures ranging from 20 to 30 °C ± 0.1 °C.

5.19.4. Test method

5.19.4. 1 Determine the water content following § 5.14.

5.19.4. 2 Calculate the dry weight of the sample in grams, M, required to prepare 240 g of the test solution as follows:

- M = 100 A / (100 - B)

where:

A = desired dry mass of the sample in g, and

B = the water content of the sample in %.

5.19.4.3. Calculate the quantity of distilled water as follows:

- V = 240 - S

where:

V = volume of distilled water in mL and

S = mass of the sample in g.

5.19.4.4. Add the quantity of water calculated in the jar. Position of the stirrer must allow a minimal clearance between the stirrer and the bottom of the container.

5.19.4.5. Begin stirring and to slowly add the carboxymethylcellulose. Adjust stirring speed to approximately 900 ± 100 r/min and mix for 2 hrs. Do not allow the stirring speed exceed 1,200 r/min as higher speeds tend to affect the viscosity of certain carboxymethylcellulose solutions.

NOTE: If the sample is added too quickly, an agglomeration will occur, which could prevent the complete dissolution of the sample in the indicated time interval.

5.19.4.6. Remove the stirrer and transfer the container containing the sample to the water bath until a constant temperature is reached (approximately one hour). Check the temperature of the sample with a thermometer at the end of one hour and make sure that the test temperature has been reached.

5.19.4.7. Remove the container containing the sample from the water bath and stir vigorously for 10 sec. Measure viscosity with the Brookfield viscometer, choosing the spindle and speed following table 1. Let the spindle turn for three minutes before carrying out the reading.

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5.19.5. Calculation

28.1 Calculate viscosity, V, in millipascals per second (mP/s) as follows:

- V = reading × factor

5.19.6. Expression of results

Express the result of Brookfield viscosity at 25 °C by indicating the concentration of the solution, the spindle, and the spindle speed used.

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