SODIUM CARBOXYMETHYL CELLULOSE, ENZYMATICALLY HYDROLYZED

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding tentative specifications prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997). This substance was included at the 51st JECFA (1998) in the group ADI "not specified" for modified celluloses, established at the 35th JECFA in 1989.

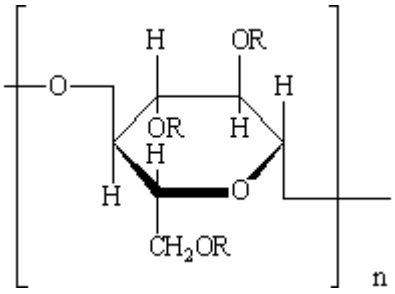
SYNONYMS Enzymatically hydrolyzed carboxymethyl cellulose; CMC-ENZ; INS No. 469

DEFINITION The product is the sodium salt of a carboxymethyl ether of cellulose, which has been partially hydrolyzed by enzymatic treatment with food-grade *Trichoderma reesei* cellulase. The total content of mono- and disaccharides does not exceed about 7.5%.

Chemical names: Carboxymethyl cellulose, sodium, partially enzymatically hydrolyzed

Chemical formula: Sodium salts of polymers containing substituted anhydroglucose units with the general formula:
$[C_6H_7O_2(OH)_x(OCH_2COONa)_y]_n$
where
n is the degree of polymerization
x = 1.50 to 2.80
y = 0.2 to 1.50
x + y = 3.0
(y = degree of substitution)

Structural formula:



where R = H, CH_2COONa or CH_2COOH

Formula weight: 178.14, where y = 0.20
282.18, where y = 1.50
Macromolecules: Not less than 800 (n about 4)

Assay: Not less than 99.5%, including mono- and disaccharides, on the dried basis

DESCRIPTION White or slightly yellowish or greyish, odourless, slightly hygroscopic granular or fibrous powder

FUNCTIONAL USES Carrier, glazing agent, stabilizer, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
Foam test	Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxymethyl cellulose, whether hydrolysed or not, from other cellulose ethers and from alginates and natural gums.
Precipitate formation	To 5 ml of a 0.5% solution of the sample add 5 ml of a 5% solution of copper or aluminium sulfate. A precipitate appears. This test distinguishes sodium carboxymethyl cellulose, whether hydrolysed or not, from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum.
Colour reaction	Add 0.5 g of the powdered sample to 50 ml of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. Dilute 1 ml of the solution with 1 ml of water in a small test tube. Add 5 drops of 1-naphthol TS. Incline the tube, and carefully introduce down the side of the tube 2 ml of sulphuric acid so that it forms a lower layer. A red-purple colour develops at the interface.
Viscosity (60% solids)	Not less than 2500 mPas corresponding to an average molecular weight of 5000 D. This test also distinguishes enzymatically hydrolyzed CMC from non-hydrolyzed CMC since it is not possible to make a 60% solution of ordinary CMC. See description under TESTS

PURITY

Loss on drying (Vol. 4)	Not more than 12% (105° to constant weight)
pH (Vol. 4)	Not less than 6.0 and not more than 8.5 (1 in 100 solution)
Sodium chloride and sodium glycolate	Not more than 0.5%, singly or in combination See descriptions under TESTS
Degree of substitution	Not less than 0.2 and not more than 1.50 carboxymethyl groups (CH_2COOH) per anhydroglucose unit on the dried basis See description under TESTS
Residual enzyme activity	Passes test See description under TESTS
Lead (Vol. 4)	Not more than 3 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

IDENTIFICATION TESTS

Viscosity (60% solids)

To 40 ml of water, add 60 g of the sample, stirring continuously. To ensure the formation of a clear solution without air bubbles, leave the solution to stand in a refrigerator (+4°) for several hours. Using a Bohlin viscometer or an equivalent instrument, measure the viscosity of the solution at 25° using a shear rate of 147sec^{-1}.

PURITY TESTS

Sodium chloride

Heat 5 g of the sample, weighed to the nearest 0.1 mg, in a platinum or porcelain crucible, first with a small flame so that the sample does not ignite and then, when the charring is complete, heat further in an electric oven for 15 min at about 500°. After cooling, pulverize the ashes thus obtained and extract several times with warm water. Filter the extracts into a 500-ml volumetric flask, acidify with nitric acid and dilute to the mark with water. Determine the NaCl content of 100 ml of this extract by the method of Volhard, using 0.2 N silver nitrate and 0.02 N ammonium thiocyanate. Each ml of 0.02 N silver nitrate is equivalent to 1.169 mg of NaCl. Calculate the sodium chloride content by the formula:

$$\% \text{ NaCl} = [(a \times 0.001169 \times 5)/b] \times 100$$

where
a = 0.02 N silver nitrate consumed (ml)
b = dry weight of the sample (g)

Sodium glycolate

Proceed as directed under *Chromatography (High Performance Liquid Chromatography)* using the following conditions and using pure glycolic acid as the reference substance:
Column: Two cation exchange columns in the H^+-form in series, e.g. two Fast Fruit Juice columns, 15 cm x 7.8 mm, Waters, or equivalent
Elution: Isocratic
Mobile phase: Aqueous 0.05% phosphoric acid
Flow: 0.5 ml/min
Detector type: UV or diode array, 205 nm
Sample size: 50 µl of a solution of 200.0 mg of the sample in 20 ml of eluent

Degree of substitution

Weigh accurately about 200 mg of the sample, previously dried at 105° to constant weight, and transfer it into a 250-ml glass-stoppered Erlenmeyer flask. Add 75 ml of glacial acetic acid, and connect the flask with a water-cooled condenser, and reflux gently on a hot plate for 2 h. Cool, transfer the solution to a 250-ml beaker with the aid of 50 ml of glacial acetic acid, and titrate with 0.1 N perchloric acid in dioxane while stirring with a magnetic stirrer. Determine the endpoint potentiometrically with a pH meter equipped with a standard glass electrode and a calomel electrode modified as follows: Discard the aqueous potassium chloride solution, rinse and fill with the supernatant liquid obtained by shaking thoroughly 2 g each of potassium chloride and silver chloride (or silver oxide) with 100 ml of methanol, then add a few crystals of potassium chloride and silver chloride (or silver oxide) to the electrode.

Record the ml of 0.1 N perchloric acid versus mV (0 to 700 mV range), and continue the titration to a few ml beyond the endpoint. Plot the titration curve, and read the volume (A), in ml, of 0.1 N perchloric acid at the

inflection point.

Calculate the degree of substitution (DS) by the formula

(16.2 A/G)/[1.000 - (8.0 A/G)],

where
A = the volume of 0.1 N perchloric acid required (ml)
G = weight of the sample taken (mg)
16.2 = one-tenth of the formula weight of one anhydroglucose unit
8.0 = one-tenth of the formula weight of one sodium carboxymethyl group

Residual enzyme activity

Prepare a 5% solution of sodium carboxymethyl cellulose having a viscosity of 25-50 mPas as a 2% solution. To 20 g of this 5%-solution, add 2 g of a 20% aqueous solution of the sample. Using a Bohlin viscometer or an equivalent instrument, follow the viscosity of the mixture for 10 minutes at 25^{o}, using a shear rate of 147sec^{-1}. No change in viscosity (indicating hydrolysis of the sodium carboxymethyl cellulose), occurs. As a control, measure the viscosity of 2 g of water and 20 g of the same sodium carboxymethyl cellulose solution.

METHOD OF ASSAY

Calculate the percentage of enzyme treated sodium carboxymethyl cellulose by subtracting from 100 the sum of the percentages of sodium chloride and sodium glycolate, determined separately by the procedures above.