

CARRAGEENAN

Prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). A group ADI "not specified" for carrageenan and processed *eucheuma* seaweed was established at the 57th JECFA (2001).

SYNONYMS

Irish moss gelose (from *Chondrus* spp.); Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); INS No. 407.

DEFINITION

A substance with hydrocolloid properties obtained from certain members of the class *Rhodophyceae* (red seaweeds).

The principal commercial sources of carrageenans are the following families and genera of the class of *Rhodophyceae*:

Furcellariaceae such as *Furcellaria*

Gigartineae such as *Chondrus*, *Gigartina*, *Iridaea*

Hypnaeaceae such as *Hypnea*

Phylloporaceae such as *Phyllophora*, *Gymnogongrus*, *Ahnfeltia*

Solieriaceae such as *Eucheuma*, *Anatheca*, *Meristotheca*.

Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α -1,3 and β -1,4 in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent polysaccharides in carrageenan are designated as kappa-, iota-, and lambda-carrageenan. Kappa-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; iota-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between kappa-carrageenan and iota-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In lambda-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).

Carrageenan is obtained by extraction from seaweed into water or aqueous dilute alkali. Carrageenan may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol. Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes.

C.A.S. number

9000-07-1

DESCRIPTION

Yellowish or tan to white, coarse to fine powder that is practically odourless.

FUNCTIONAL USES

Thickener, gelling agent, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4)	Insoluble in ethanol; soluble in water at a temperature of about 80°, forming a viscous clear or slightly opalescent solution that flows readily; disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.
<u>Test for sulfate</u>	Dissolve a 100-mg sample in 20 ml of water (with heating if necessary), and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS; filter if a precipitate forms. Boil the solution or the filtrate for 5 min. A white, crystalline precipitate appears.
<u>Test for galactose and anhydrogalactose</u> (Vol. 4)	Proceed as directed under Gum Constituents Identification (Vol. 4) using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.
<u>Identification of hydrocolloid and predominant type of copolymer</u>	<p>Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel. To 50 ml of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool.</p> <p>A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa type, and a compliant ("elastic") gel indicates a predominantly iota type. If the solution does not gel, the carrageenan is of a predominantly lambda type.</p>
<u>Infrared absorption</u>	Passes test See description under TESTS
PURITY	
<u>Loss on drying</u> (Vol. 4))	Not more than 12% (105° to constant weight)
<u>pH</u> (Vol. 4)	Between 8 and 11 (1 in 100 suspension)
<u>Viscosity</u>	Not less than 5 cp at 75° (1.5% solution) See description under TESTS
<u>Sulfate</u>	Not less than 15% and not more than 40% (as SO ₄ ²⁻) on a dry weight basis See description under TESTS
<u>Total ash</u>	Not less than 15% and not more than 40% on a dry weight basis See description under TESTS.
<u>Acid-insoluble ash</u> (Vol. 4)	Not more than 1%
<u>Acid-insoluble matter</u> (Vol. 4)	Not more than 2% Use 2 g of sample obtained from part (a) of the procedure for sulfate determination.
<u>Residual solvents</u>	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in

combination
See description under TESTS

Microbiological criteria
(Vol. 4)

Initially prepare a 10^{-1} dilution by adding a 50-g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender.
Total (aerobic) plate count: Not more than 5000 cfu/g
Salmonella spp.: Negative per test
E. coli: Negative in 1 g

Arsenic (Vol. 4)

Not more than 3 mg/kg
Determine by atomic absorption hydride technique using a 3 gram sample.

Lead (Vol. 4)

Not more than 2 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."

Cadmium

Not more than 2 mg/kg
See description under TESTS

Mercury

Not more than 1 mg/kg
See description under TESTS

TESTS

IDENTIFICATION TESTS

Infrared absorption

Obtain infrared absorption spectra on the gelling and non-gelling fractions of the sample by the following procedure:

Disperse 2 g of the sample in 200 ml of 2.5% potassium chloride solution, and stir for 1 h. Let stand overnight, stir again for 1 h, and transfer into a centrifuge tube. (If the transfer cannot be made because the dispersion is too viscous, dilute with up to 200 ml of the potassium chloride solution.) Centrifuge for 15 min at approximately 1000 x g.

Remove the clear supernatant, resuspend the residue in 200 ml of 2.5% potassium chloride solution, and centrifuge again. Coagulate the combined supernatants by adding 2 volumes of 85% ethanol or isopropanol (NOTE: Retain the sediment for use as directed below). Recover the coagulum, and wash it with 250 ml of the alcohol. Press the excess liquid from the coagulum, and dry it at 60° for 2 h. The product obtained is the non-gelling fraction (lambda-carrageenan).

Disperse the sediment (retained above) in 250 ml of cold water, heat at 90° for 10 min, and cool to 60°. Coagulate the mixture, and then recover, wash, and dry the coagulum as described above. The product obtained is the gelling fraction (kappa- and iota-carrageenan).

Prepare a 0.2% aqueous solution of each fraction, cast films 0.5 mm thick (when dry) on a suitable non-sticking surface such as Teflon, and obtain the

infrared absorption spectrum of each film. (Alternatively, the spectra may be obtained using films cast on potassium bromide plates, if care is taken to avoid moisture).

Carrageenan has strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. Absorption maxima are 1065 and 1020 cm^{-1} for gelling and non-gelling types, respectively. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave number (cm^{-1})	Molecular Assignment	Absorbance relative to 1050 cm^{-1}		
		Kappa	Iota	Lambda
1220-1260	ester sulfate	0.2-1.2	1.2-1.6	1.4-2.0
928-933	3,6-anhydrogalactose	0.2-0.6	0.2-0.4	0-0.2
840-850	galactose-4-sulfate	0.1-0.5	0.2-0.4	-
825-830	galactose-2-sulfate	-	-	0.2-0.4
810-820	galactose-6-sulfate	-	-	0.1-0.3
800-805	3,6-anhydrogalactose-2-sulfate	0-0.2	0.2-0.4	-

PURITY TESTS

Sulfate

Principle

Hydrolysed sulfate groups are precipitated as barium sulfate.

Procedure

(a) Disperse an accurately weighed 8 g sample of commercial product into 400 ml of 60% w/w isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 10-ml portions of 60% isopropanol/water. Dry the material at 105° to constant weight. Approximately 1 g of the dried matter is to be used for part (b). The remainder should be retained for determination of Total ash and Acid-insoluble matter.

(b) Accurately weigh a 1 g sample (W_1) obtained from part (a). Transfer the sample to a 100-ml long-neck round-bottom flask. Add 50 ml of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 ml of a 10% (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear. Transfer the solution to a 600-ml beaker, bring to a boil, and add dropwise 10 ml of a 10% barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash-free slow-filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800° in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator.

Weigh the crucible containing the ash. Calculate the percentage sulfate from the weight in g (W_2) of the ash (barium sulfate) using the formula:
 $(W_2/W_1) \times 100 \times 0.4116$

Total ash

Accurately weigh 2 g of the dried sample (W_1) obtained from part (a) under

the procedure for sulfate determination above. Transfer to a previously ignited, tared silica or platinum crucible. Heat the sample with a suitable infrared lamp, increasing the intensity gradually, until the sample is completely charred; continue heating for an additional 30 min. Transfer the crucible with the charred sample into a muffle furnace and ignite at about 550° for 1 h. Cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight (W_2) is obtained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 1-in-10 solution of ammonium nitrate and dry under an infrared lamp. Repeat the ignition step.

Calculate the percentage of total ash of the sample:

$$(W_2/W_1) \times 100$$

Retain the ash for the Acid-insoluble ash test.

Viscosity

Transfer 7.5 g of the sample into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in a water bath with continuous agitation, until a temperature of 80° is reached (20 - 30 min). Add water to adjust for loss by evaporation, cool to 76-77°, and heat in a constant temperature bath at 75°. Pre-heat the bob and guard of a Brookfield LVF or LVT viscometer to approximately 75° in water. Dry the bob and guard, and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent. (Note. Samples of some types of carrageenan may be too viscous to read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle and take the reading on the 0-100 scale or on the 0-500 scale.)

Record the results in centipoises, obtained by multiplying the reading on the scale by the factor given by the Brookfield manufacturer.

Residual solvents

Standard Alcohol Solution

Transfer 500 mg each of chromatographic quality methanol, ethanol, and isopropanol into a 50 ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water and mix.

TBA Standard Solution

Transfer 500 mg of chromatographic quality tertiary-butyl alcohol into a 50-ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water and mix.

Mixed Standard Solution

Pipet 4 ml each of the Standard Alcohol Solution and of the TBA Standard Solution into a 125-ml graduated Erlenmeyer flask, dilute to about 100 ml with water, and mix. This solution contains approximately 40 µg of each alcohol per ml.

Sample Preparation

Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distil about 100 ml, adjusting the heat so that foam does not enter the column. Add 4.0 ml of TBA Standard Solution to the distillate to obtain the Sample Preparation.

Procedure

Inject 5 μ l of the Mixed Standard Solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m x 3.2-mm stainless steel column packed with 80/100-mesh Porapak QS or equivalent. The carrier is helium flowing at 80 ml per min. The injection port temperature is 200°; the column temperature is 165°; and the detector temperature is 200°. The retention time of isopropanol is about 2 min, and that of tertiary-butyl alcohol about 3 min.

Measure the areas of the methanol, ethanol, isopropanol, and TBA peaks. Calculate each response factor, f_i , by the formula A_i / A_{TBA} , in which A_i is the area of each alcohol peak (I = methanol, ethanol, or isopropanol). Similarly, inject 5 μ l of the Sample Preparation, and measure the peak areas, recording the area of each alcohol peak as A_i , and that of the tertiary-butyl alcohol peak as A_{TBA} .

Calculate each alcohol content, in mg/kg, in the sample taken by the formula:

$$A_i \cdot 4000 / f_i \cdot A_{TBA} \cdot W$$

where W is the weight of the sample taken (grams).

Lead (Vol. 4)

Principle

The sample is wet-ashed with nitric and perchloric acids and analysed using flame atomic absorption spectrophotometry (Volume 4).

Equipment

Atomic absorption spectrophotometer

Reagents

Nitric acid, concentrated, Reagent Grade
Perchloric acid, concentrated, Reagent Grade
Hydrochloric acid, concentrated, Reagent Grade
Lead standard solution (certified)

Solutions

Stock solution (1 mg/ml): Dilute an appropriate volume of certified reagent lead standard solution with distilled and deionized water (D/D water) to make one liter.

Intermediate solutions: (a) 100 μ g/ml. Pipet 10 ml of the stock solution into a 100-ml volumetric flask and dilute to volume with D/D water. (b) 10 μ g/ml. Pipet 10 ml of the 100 μ g/ml solution into a 100-ml volumetric flask and dilute to volume with D/D water.

Working solutions: Assemble four 100-ml volumetric flasks and transfer to them (pipet), respectively, 1, 5, 10, and 20 ml of intermediate lead solution (b). Dilute to volume with D/D water to make solutions containing 0.1, 0.5, 1, and 2 µg Pb/ml.

Sample preparation

(CAUTION: This procedure employs concentrated oxidizing acids and results in evolution of noxious gases. Perform operations in a fume hood.)

Accurately weigh 7.5 grams of a representative dry powdered test sample into a 250-ml Erlenmeyer flask. Set up a reagent blank and carry through the same operations as performed on the test sample. Wet the test sample with ca. 10 ml of D/D water and add 25 ml of nitric acid. Heat gently on a hot plate (100° - 150°) until most of the dark fumes are evolved (ca. one hour); swirl the flask occasionally. Cool and add 5 ml of perchloric acid; particles become visible at this stage. Heat gently (hot plate, 100° - 150°) to concentrate until the solution turns yellowish or colourless (ca. one hour). Midway during the heating, if the solution darkens, slowly add 2-3 ml portions of nitric acid as necessary until the desired colour is achieved; do not let the solution go to dryness. Cool the digest and wash the sides of the flask with ca. 5 ml of D/D water and swirl. Add 2 ml of hydrochloric acid. Heat again until all brown fumes are evolved and the solution is white to yellowish in colour; do not let the solution go to dryness. Cool the solution and wash the sides of the flask with ca. 10 ml of D/D water. Transfer the slightly viscous solution to a 50-ml volumetric flask and dilute to volume with D/D water. Filter using two layers of filter paper (Whatman no. 5 or equivalent).

Determination

Set the spectrophotometer to previously established optimum conditions at 283.3 nm using an air/acetylene oxidizing flame. Measure the absorbance of the sample, blank, and working solutions. Prepare a standard curve by plotting absorbance against µg Pb/ml for the blank and working solutions. Determine the concentration of lead in the sample solution from the standard curve.

The concentration of lead in the test sample (mg Pb/kg) is:

$$[\text{Pb}] = F \times A/B$$

where A is the concentration of lead in the sample solution (µg/ml), B is the weight of the test sample (grams), and F is the dilution factor (50 ml).

Cadmium (Vol. 4)

Proceed as directed above for the determination of lead, using 228.8 nm as the analysis wavelength. Intermediate and working solutions are prepared from certified reagent cadmium standard solution:

Intermediate solutions: (a) 100 µg/ml. Pipet 10 ml of the stock solution (1mg/ml) into a 100-ml volumetric flask and dilute to volume with distilled and deionized (D/D) water. (b) 10 µg/ml. Pipet 10 ml of solution (a) into a 100-ml volumetric flask; dilute to volume with D/D water. (c) 1 µg/ml. Pipet 1 ml of solution (a) into a 100-ml volumetric flask; dilute to volume with D/D water.

Working solutions: Assemble five 50-ml volumetric flasks and transfer to them (pipet), respectively, 0.5, 2.5, 5.0, 10, and 20 ml of intermediate solution (c). Dilute to volume with D/D water to make solutions containing 0.01, 0.05, 0.1, 0.20, and 0.40 µg Cd/ml.

The concentration of cadmium in the test sample (mg Cd/kg) is:
 $[Cd] = F \times A/B$

where A is the concentration of cadmium in the sample solution ($\mu\text{g/ml}$), B is the weight of the test sample (grams), and F is the dilution factor (50 ml).

Mercury (Vol. 4)

Principle

The sample is wet-ashed with nitric and perchloric acids and analysed using hydride-generation atomic absorption spectrophotometry (Volume 4).

Equipment

Atomic absorption spectrophotometer equipped with a hydride vapour generator. Integral to the generator is a reactor tube or coil and a peristaltic pump with dual tubing channels: one channel for the sample solution and one for the two reagent solution tubes. Flow control is determined by tubing size and tubing clamps. Flow rates are measured at the exit of the hydride generator.

Reagents

Nitric acid, concentrated, Reagent Grade
Perchloric acid, concentrated, Reagent Grade
Hydrochloric acid, concentrated, Reagent Grade
Sodium borohydride, >98%
Sodium hydroxide, Reagent grade
Mercury standard solution (certified)

Solutions

Nitric acid-perchloric acid (1:1): Mix equal volumes of the two acids.
Hydrochloric acid, 5M: Dilute 417 ml concentrated hydrochloric acid to 1 liter with deionized water.

Sodium borohydride solution, 0.4% (Prepare immediately before use.): First, dissolve 2.5 g sodium hydroxide in deionized water. Then, add and dissolve 2.0 g sodium borohydride. Dilute to 500 ml.

Stock solution (1 mg/ml): Dilute an appropriate volume of certified reagent mercury standard solution with distilled and deionized water (D/D water) to make one liter.

Intermediate solutions: (a) 10,000 $\mu\text{g/l}$. Pipet 1 ml of the stock solution into a 100-ml volumetric flask and dilute to volume with D/D water. (b) 100 $\mu\text{g/l}$. Pipet 1 ml of the 10,000 $\mu\text{g/l}$ solution into a 100-ml volumetric flask and dilute to volume with D/D water.

Working solutions: Assemble five 100-ml volumetric flasks and transfer to them (pipet), respectively, 1, 5, 10, 15, and 20 ml of intermediate solution (b). To each, add 10 ml of 1:1 nitric acid-perchloric acid and dilute to volume with D/D water to make solutions containing 1, 5, 10, 15, and 20 $\mu\text{g Hg/l}$.

Sample preparation

(CAUTION: This procedure employs concentrated oxidizing acids and results in evolution of noxious gases. Perform operations in a fume hood.)

Accurately weigh 5 grams of a representative dry powdered test sample into a 250 ml Erlenmeyer flask. Set up a reagent blank and carry through the

same operations as performed on the test sample. Wet the test sample with 5 ml of D/D water and then add 10 ml of 1:1 nitric acid-perchloric acid. Heat gently on a hot plate (100o-150o) until all of the dark fumes are evolved and the solution turns yellowish or colourless; swirl the flasks occasionally. Do not let the solution go to dryness. Cool and wash the sides of the flask with a small amount of D/D water. (Some particles may be visible.) Cover the flask lightly and let the slightly viscous solution stand overnight. Transfer the solution to a 50-ml volumetric flask and dilute to volume with D/D water. Filter using 2 layers of Whatman no. 5 (or equivalent) filter paper into a 100-ml Erlenmeyer flask. Immerse the flask in an ultrasonic bath and sonicate it for 10 minutes or until bubbles no longer form on the surface of the solution.

Determination

Calibrate (using water) the peristaltic pump to provide a flow rate of the sample solution of 8 ml/min and a combined flow rate for the two reagent solutions (sodium borohydride and 5M hydrochloric acid) of 2 ml/min. (The combined flow rate is achieved with a single pump setting.)

Set the spectrophotometer to previously established optimum conditions at the mercury lamp wavelength of 253.7 nm.

Transfer suitable quantities of the two reagent solutions into separate graduated cylinders. Insert separate aspirator tubing leading from the peristaltic pump into each reagent solution and into the sample flask. Start the flow of argon carrier gas (tank outlet pressure: 3.2 ± 0.2 kg/cm²) through the hydride vapour generator of the spectrophotometer. Start the pump to initiate flow of the three solutions into the hydride generator manifold where they are mixed and pass into the reactor coil to generate atomic mercury, which is carried into the absorbance cell of the spectrophotometer. Measure the absorbance for the sample. Repeat for the blank solution and each of the working standards.

Prepare a standard curve by plotting absorbance against $\mu\text{g Hg/l}$ for the blank and working solutions. Determine the concentration of mercury in the sample solution from the standard curve.

The concentration of mercury in the test sample (mg Hg/kg) is:

$$[\text{Hg}] = FxA/1000B$$

where A is the concentration of mercury in the sample solution ($\mu\text{g/l}$), B is the weight of the test sample (grams), and F is the dilution factor (50 ml).