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Carrageenan

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CAS RN®: 9000-07-1.

DEFINITION

Carrageenan is the hydrocolloid obtained by extraction with water or aqueous alkali, from some members of the class Rhodophyceae (red seaweeds). Carrageenan consists chiefly of potassium, sodium, calcium, magnesium, and ammonium sulfate esters of galactose and 3,6-anhydrogalactose copolymers. These hexoses are alternately linked α-1,3 and β-1,4 in the polymer. The prevalent copolymers in the hydrocolloid are designated 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 copolymer 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). The ester sulfate content for Carrageenan ranges from 18% to 40%. In addition, it contains inorganic salts that originate from the seaweed and from the process of recovery from the extract.

Carrageenan is recovered by alcohol precipitation, by drum drying, or by freezing. The alcohols used during recovery and purification are restricted to methanol, alcohol, and isopropyl alcohol. Carrageenan that is recovered by drum-roll drying may contain mono- and diglycerides or up to 5% of polysorbate 80 used as roll-stripping agents.

IDENTIFICATION

• **A.**

Sample solution: A 20-mg/mL solution prepared by heating a uniform dispersion in a hot water bath to 80°

Analysis: Cool the *Sample solution*.

Acceptance criteria: The *Sample solution* becomes more viscous upon cooling and may form a gel.

Change to read:

• **B.**

Sample solution: Prepare as directed for the *Sample solution* in *Identification A*.

Analysis: To 10 mL of the *Sample solution*, while still hot, add 4 drops of a 0.1-g/mL solution of potassium chloride, mix, and cool.

Acceptance criteria: ▲A short-textured (“brittle”) gel that has hardness and rigidity but breaks or shatters readily with a relatively smooth fracture indicates a carrageenan of a predominantly kappa type; a compliant (“elastic”) gel that does not break or shatter, but recovers its original shape and dimensions after the removal of deformation load indicates a predominantly iota type.▲ (NF 1-May-2024) If the solution does not gel, the carrageenan is of a predominantly lambda type.

• **C.**

Analysis: Dilute a portion of the *Sample solution*, prepared as directed in *Identification A*, with 4 parts of water, and add 2–3 drops of methylene blue TS.

Acceptance criteria: A blue, stringy precipitate is formed (also positive for furcellaran, a similar colloid).

Change to read:

• **D.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*

[Note—Perform *Procedure B* if *Procedure A* is not working for the sample preparation.]

Procedure A▲ (NF 1-May-2024)

Sample 1: Disperse 2 g in 200 mL of a ▲50-mg/mL▲ (NF 1-May-2024) solution of potassium chloride, and stir for 1 h. Allow to stand for 18 h, stir again for 1 h, and transfer to 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 at approximately 1000 × *g* for 15 min.

Remove the clear supernatant, resuspend the residue in 200 mL of a 25-mg/mL solution of potassium chloride, and centrifuge again. Coagulate the combined supernatants by adding 2 volumes of dilute alcohol (9 in 10). (Retain the sediment for use in preparing *Sample 2*.) Recover the coagulum, and wash with 250 mL of the dilute alcohol. Press the excess liquid from the coagulum, and dry it at 60° for 2 h. The material so obtained is the nongelling fraction (lambda carrageenan).

Sample 2: Disperse the sediment retained from the preparation of *Sample 1* in ▲100 mL▲ (NF 1-May-2024) of cold water, heat at 90° for 10 min, and cool to 60°. Coagulate the mixture, then recover, wash, and dry the coagulum as described above. The material so obtained is

the gelling fraction (kappa- and iota-carrageenan).

Analysis: Prepare a 2-mg/mL solution of each *Sample*, and cast films 5 µm thick (when dry) on a suitable nonsticking surface, and obtain the IR absorption spectrum of each film.

▲**Procedure B**

Sample stock solution: Prepare a 2-g/L solution of the carrageenan sample to be examined.

Sample solution: Transfer 4.5 mL of the *Sample stock solution* into a plastic flat-bottom weighing boat about 35 mm in diameter and allow to dry completely in an airflow oven at 60° until a film of about 10 µm thick is obtained (about 16 h).

Analysis: Obtain the IR absorption spectrum of film prepared using either *Procedure A* or *Procedure B* (see *Mid-Infrared Spectroscopy ⟨854⟩*). [NOTE—Perform instrument background correction and noise smoothing using instrument software based on the sample spectrum acquired.]▲ (NF 1-MAY-2024)

Acceptance criteria: Carrageenan has strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. ▲Other characteristic absorption bands and their intensities relative to the maximal absorbance at 1065–1075 cm^{-1} are as shown in *Table 1*.

Table 1

Wave Number (cm^{-1})	Molecular Assignment	Absorbance Relative to the Absorbance at the Peak Between 1065–1075 cm^{-1}		
		Kappa	Iota	Lambda
1220–1260	Ester sulfate	0.5–1.8	1.0–1.6	2.2–3.3
926–938	3,6-Anhydro-D-galactose	0.2–0.6	0.2–0.5	0–0.2
838–850	Galactose-4-sulfate	0.2–0.5	0.1–0.4	—
825–830	Galactose-2-sulfate	—	—	0.3–0.7
810–820	Galactose-6-sulfate	—	—	0.2–0.6
800–805	3,6-Anhydro-D-galactose-2-sulfate	0.0–0.1	0.1–0.4	—

▲ (NF 1-May-2024)

IMPURITIES

• **ARSENIC ⟨211⟩, *Procedures, Procedure 1*:** NMT 3 ppm

• **LEAD ⟨251⟩, *Procedures, Procedure 1*:** NMT 10 ppm

SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** The total bacterial count does not exceed 200 cfu/g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

• **LOSS ON DRYING ⟨731⟩**

Analysis: Dry a sample at a pressure not exceeding 10 mm of mercury at 70° for 18 h, cool in a desiccator, and weigh.

Acceptance criteria: It loses NMT 12.5% of its weight.

• **SOLUBILITY IN WATER:** NMT 30 mL of water is required to dissolve 1 g at a temperature of 80°.

• **ACID-INSOLUBLE MATTER**

Sample: 2 g

Analysis: Transfer the *Sample* to a 250-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. Cover with a watch glass, and heat on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod, and replacing any water lost by evaporation. Transfer 500 mg of a suitable filter aid to the beaker, and filter through a tared filtering crucible provided with a 2.4-cm glass fiber filter. Wash the residue several times with hot water, dry at 105° for 3 h, cool in a desiccator, and weigh. The difference between the total weight and the sum of the weights of the filter aid, crucible, and glass fiber filter is the weight of the acid-insoluble matter.

Acceptance criteria: NMT 2.0% of the weight of Carrageenan taken

• **VISCOSITY–ROTATIONAL METHODS ⟨912⟩**

Sample: 7.5 g

Analysis: Transfer the *Sample* to a tared, tall-form, 600-mL beaker, add 450 mL of water, and disperse with agitation for 15 min. Add water to bring the weight to 500 g, and heat in a water bath, with continuous agitation, until a temperature of 80° is reached. Add water to adjust for loss by evaporation, cool to between 76° and 77°, and place in a constant-temperature bath maintained at 75°. Provide a suitable rotational

viscometer with a spindle 1.88 cm in diameter and 6.51 cm high, using an immersion depth of 8.10 cm (No. 1 spindle). Allow the spindle to rotate in the solution at 30 rpm for 6 revolutions, then observe the scale reading. Convert the scale reading to centipoises by multiplying by the constant for the spindle and speed used.

Acceptance criteria: At 75° is NLT 5 centipoises.

Change to read:

• **Articles of Botanical Origin** ⟨561⟩, *Methods of Analysis, Total Ash*: NMT 35.0%

▲[Note—Take the air-dried sample if necessary for the total ash test.]▲ (NF 1-May-2024)

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, preferably in a cool place.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CARRAGEENAN	Documentary Standards Support	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

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