GELLAN GUM

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SYNONYM INS No. 418

DEFINITION Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Pseudomonas elodea*, purified by recovery with ethanol or 2-propanol, dried, and milled. The high molecular weight polysaccharide is principally composed of a tetrasaccharide repeating unit of one rhamnose, one glucuronic acid, and two glucose units, and is substituted with acyl (glyceryl and acetyl) groups as the O-glycosidically-linked esters. The glucuronic acid is neutralized to a mixed potassium, sodium, calcium, and magnesium salt. It usually contains a small amount of nitrogen containing compounds resulting from the fermentation procedures.

C.A.S. number 71010-52-1

Formula weight Approximately 500,000

Assay Yields, on the dried basis, not less than 3.3% and not more than 6.8% of carbon dioxide (CO_2).

DESCRIPTION Off-white powder

FUNCTIONAL USES Thickener, gelling agent, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, forming a viscous solution; insoluble in ethanol

Gel test with calcium ion Add 1.0 g of the sample to 99 ml of water, and stir for about 2 h, using a motorized stirrer having a propeller-type stirring blade. Draw a small amount of this solution into a wide bore pipet and transfer into a 10% solution of calcium chloride. A tough worm-like gel will be formed immediately.

Gel test with sodium ion Add 1.0 g of the sample to 99 ml of water, and stir for about 2 h, using a motorized stirrer having a propeller-type stirring blade. Add 0.50 g of sodium chloride, heat to 80° with stirring, and hold at 80° for 1 min. Allow the solution to cool to room temperature. A firm gel is formed.

PURITY

Loss on drying (Vol. 4) Not more than 15% (105°, 2½ h)

Nitrogen (Vol. 4)	Not more than 3%
Residual solvents	Not more than 50 mg/kg of ethanol; not more than 750 mg/kg of 2-propanol See description under TESTS
Microbiological criteria	Total plate count: Not more than 10,000 cfu/g *E. coli:* Negative by test *Salmonella*: Negative by test Yeasts and moulds: Not more than 400 cfu/g See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Residual solvents

Standard solutions

Transfer 100.0 mg of chromatographic quality ethanol into a 200-ml volumetric flask and 150.0 mg of 2-propanol into a 20-ml volumetric flask, then dilute to volume with water. Pipet each 1 ml of solutions into a 100-ml volumetric flask and dilute to volume with water as standard solution A. Pipet 10 and 5 ml of standard A into two separate 20-ml volumetric flasks and dilute to volume with water as standard solution B and standard solution C.

Chromatography conditions

Column: 25% diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm i.d. with 1.4 µm-film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas: Helium
Flow rate: 1.8 ml/min
Detector: Flame ionization detector (FID)
Temperatures:
- Injection port: 250°
- Oven: Hold for 5 min at 40°, then 40° to 92° at 4°/min
- Detector: 260°

The retention times of ethanol and 2-propanol are about 6.5 and 7.5 min, respectively.

Samples

Weigh accurately 0.10 g of the sample into each of four 20 ml head-space vials. Add a magnetic stirring bar and 10 ml of either water, standard solution A, B or C into each vial and seal. After standing vials overnight at room temperature, stir the solution in the vials for 1 min.

Procedure

Place the sample vial in the sample tray on head-space gas chromatograph. Heat vials at 60° for 40 min with continuous agitation. Inject 1.0 ml of the head space gas (Syringe temperature: 100°,

Transfer temperature: 120°) in the vial into the chromatograph and measure the peak area for ethanol and 2-propanol. Plot the relationship between the added amount against the peak area for ethanol or 2-propanol. Extrapolate the x-intercept for ethanol and 2-propanol (w_e and w_p). Calculate the concentration of ethanol and 2-propanol from;

$$\text{Ethanol (mg/kg)} = w_E / W$$
$$\text{2-Propanol (mg/kg)} = w_P / W$$

Where
W is weight of sample (g).

Microbiological criteria

Total plate count

Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 12-15 ml of Plate Count Agar previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, allow the agar to solidify. Invert the plates and incubate for 48±2 h at 35±1°.

After incubation count the growing colonies visible on each plate and record the number of colonies. Take the average of both plates, and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

E. coli

Using aseptic technique, disperse 1 g of sample in 99 ml of Lactose broth using either a Stomacher, shaker or stirrer to fully dissolve the sample. Limit the dissolving time to about 15 min and then lightly seal the container and incubate the broth for 18-24 h at 35±1°. Using a sterile pipette, inoculate 1 ml of the incubate into a tube containing 10 ml GN broth. Incubate for 18-24 h and then streak any GN broths showing positive growth or gas production onto duplicate plates of Levine EMB agar. Incubate the plates for 24±2 h at 35±1° and then examine for colonies typical of *E. coli i.e.* showing strong purple growth with dark centre and a green metallic sheen sometimes spreading onto the agar. Record any typical *E. coli* colonies as presumptive positive, otherwise negative.
Streak any well isolated suspect colonies onto a plate of PCA and incubate for 18-24 h at 35±1°. Perform a Gram stain on any growth to confirm it is Gram negative. If so, disperse any colony growth into a small volume of 0.85% saline and perform chemical tests to confirm the identity of the bacterial growth. This can most conveniently be done by using API 20E or Micro ID strips or equivalent systems.

After completion of the tests, identify the organism from the Identification manual of the system used and record the final result.

Media

GN Broth (Gram Negative Broth)
Peptone 20.0 g
Dextrose 1.0 g
Mannitol 2.0 g
Sodium citrate 5.0 g
Sodium deoxycholate 0.5 g

Potassium phosphate (dibasic) 4.0 g
Potassium phosphate (monobasic) 1.5 g
Sodium chloride 5.0 g
Make up to 1 litre with distilled or de-ionised water, pH 7.0±0.2 at 25°.

Salmonella
Using aseptic technique, disperse 5 g of sample into 200 ml of sterile lactose broth using either a Stomacher, shaker or stirrer to maximise dissolution over a 15 min period. Loosely seal the container and incubate at 35±1° for 24±2 h.

Continue as per method on page 104 in Volume 4 (under "General methods, *Salmonella*). Identification can be more conveniently done using API or Micro ID systems or equivalent.

Yeasts and moulds
Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 15-20 ml of Potato dextrose Agar (either acidified or containing antibiotic) previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, and allow the agar to solidify. Invert the plates and incubate for 5 days at 20-25°.
After incubation, count the growing colonies visible on each plate using a colony counter and record the number of colonies. Separate the yeasts from the moulds according to their morphology and count them separately. Take the average of both plates and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

METHOD OF ASSAY Proceed as directed in the test for Alginates Assay (Carbon Dioxide Determination by Decarboxylation) in Volume 4 (under "Assay Methods"), using 1.2 g of the sample.