GELLAN GUM

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996). An ADI 'not specified' was established at the 37th JECFA (1990)

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 isopropyl alcohol, 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 Thickening agent, 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%

Isopropyl alcohol	Not more than 750 mg/kg See description under TESTS
Microbiological criteria	Total plate count: Not more than 10,000 colonies per gram *E. coli:* Negative by test *Salmonella*: Negative by test Yeasts and moulds: Not more than 400 colonies per gram See description under TESTS
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."

TESTS

PURITY TESTS

Isopropyl alcohol

Isopropyl alcohol (IPA) Standard Solution
Transfer 500.0 mg of chromatographic quality isopropyl 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.

Tertiary butyl alcohol (TBA) Standard Solution
Transfer 500.0 mg of chromatographic quality tert-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 IPA standard 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 each of isopropyl alcohol and of tert-butyl 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 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake the flask for 1 h, on a wrist-action mechanical shaker. Connect the flask to a fractionating column and distil about 100 ml; adjust 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 about 5 µl of the Mixed Standard Solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m x 2.3-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 165°, and the detector temperature 200°. The retention time of isopropyl alcohol is about 2 min,

and that of tert-butyl alcohol about 3 min.

Determine the areas of the IPA and TBA peaks, and calculate the response factor, f, by the formula A_{IPA}/A_{TBA}, in which A_{IPA} is the area of the isopropyl alcohol peak, and A_{TBA} is the area of the tert-butyl alcohol peak.

Similarly, inject about 5 µl of the Sample Preparation, and determine the peak areas, recording the area of the isopropyl alcohol peak as a_{IPA}, and that of the tert-butyl alcohol peak as a_{TBA}.

Calculate the isopropyl alcohol content, in mg/kg, in the sample taken by the formula:
$(a_{IPA} \times 4000)(f \times a_{TBA} \times W)$
where W is the weight of the sample taken, in 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 determination
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 determination
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 221 of *FNP 5/Rev. 2 (1991)*. 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

Processed as directed in the test for Carbon Dioxide Determination by Decarboxylation in the *General Methods*, Volume 4, using about 1.2 g of the sample weighed accurately.