CURDLAN

Prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001), superseding specifications prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999). An ADI "not specified" was established at the 57th JECFA (2001).

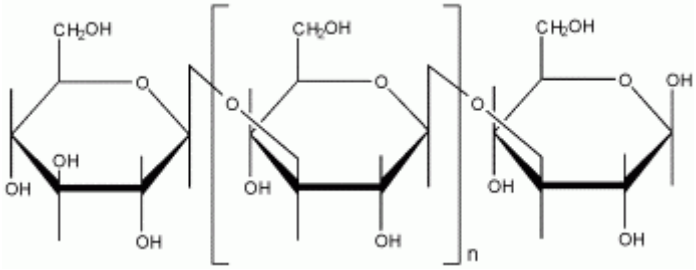
SYNONYMS Beta-1,3-glucan; INS No. 424

DEFINITION Curdlan is a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, produced by pure-culture fermentation from a non-pathogenic and non-toxicogenic strain of *Agrobacterium* biovar 1 (identified as *Alcaligenes faecalis* var. *myxogenes* at the time of discovery) or *Agrobacterium radiobacter*. Curdlan consists of β-(1,3)-linked glucose residues and has the unusual property of forming an elastic gel upon heating its aqueous suspension.

C.A.S. number 54724-00-4

Chemical formula $(C_6H_{10}O_5)_n$

Structural formula



Assay Not less than 80% (calculated as anhydrous glucose)

DESCRIPTION Odourless or almost odourless, white to nearly white powder.

FUNCTIONAL USES Firming agent, gelling agent, stabilizer, thickener.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol.

Solubility in alkali Passes test
Suspend 0.2 g of the sample in 5 ml of water, add 1 ml of 3 N sodium hydroxide, and shake. The sample dissolves.

Gel formation Heat a 2% aqueous suspension of the sample in a boiling water bath for 10 min and cool. A firm gel forms.

Precipitate formation with cupric tartrate Passes test
See description under TESTS

PURITY

Gel strength	Not less than 600 g/cm² (2% aqueous suspension) See description under TESTS
pH (Vol. 4)	6.0 - 7.5 (1% aqueous suspension)
Loss on drying (Vol. 4)	Not more than 10% (60° for 5 h, in vacuum)
Sulfated ash (Vol. 4)	Not more than 6% Test 1 g of the sample (Method I)
Nitrogen (Vol. 4)	Not more than 0.3% Test 1 g of the sample (Method II)
Lead (Vol. 4)	Not more than 0.5 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."
Microbiological criteria (Vol. 4)	Total plate count: Not more than 1,000 cfu/g E. coli: Negative in 1 g

TESTS

IDENTIFICATION TESTS

Precipitate formation with cupric tartrate	Add 5 ml of sulfuric acid TS to 10 ml of a 2% aqueous suspension of the sample, heat in a boiling water bath for 30 min and cool. Neutralize the mixture with barium carbonate. Centrifuge the mixture at 900xg for 10 min. Add 1 ml of the supernatant to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide is formed.
Gel strength	Place 200 mg of the sample into the tube of a Potter homogenizer, add 10 ml of water and homogenize at about 3,500 rpm for 5 min. Transfer the suspension into a 16 mm × 150 mm test tube, deaerate in vacuum for 3 min and heat in a boiling water bath for 10 min to form a gel. Cool the tube under running water, let stand for 30 min, then remove the gel from the tube. Cut the gel accurately at distances of 20 mm and 30 mm from the bottom to obtain a piece 10 mm long. Determine gel strength with a Rheo Meter Model CR-200D (Sun Scientific Co., Ltd., Japan; Load cell: 1,000 g) or an equivalent instrument, under the following conditions: Measurement mode: 4 Velocity of moving plate: 250 mm/min Plunger: cylindrical type, 0.5 cm diameter Read the breaking point of the gel (B). Calculate the gel strength using the following formula: Gel strength (g/cm²) = $1{,}000B/\pi r^2$ where r = the radius of the plunger (cm)

METHOD OF ASSAY Transfer about 100 mg of the sample, accurately weighed, into a 100-ml volumetric flask and dissolve in about 90 ml of 0.1 N sodium hydroxide. Add 0.1 N sodium hydroxide to volume and mix well. Transfer 5 ml of the solution into a 100-ml volumetric flask, add water to volume and mix well. Quantitatively transfer 1 ml of the solution to a small flask or test tube, add 1 ml of a 5% (w/v) solution of reagent grade phenol in water and 5 ml of sulfuric acid TS. Shake vigorously and cool in ice-cold water. Prepare a blank and a reference standard solution in the same manner, using 0.1 ml of water and 100 mg of reagent grade glucose, respectively. Determine the absorbances of the sample solution and the reference standard solution in 1-cm cells at 490 nm with a suitable spectrophotometer, using the blank solution as the blank.

Calculate the content (%) of curdlan in the sample using the following formula:

$$\text{Curdlan content (\%)} = (A/A_R) \times (0.9 \times W_R/W) \times 100$$

where
A = the absorbance of the sample solution
A_R = the absorbance of the reference standard solution
0.9 = the molecular weight of anhydrous glucose divided by the molecular weight of glucose
W = the weight of the sample (mg)
W_R = the weight of the glucose standard used as reference (mg)