POLYDEXTROSES

Prepared at the 51st JECFA (1998) and published in FNP 52 Add 6 (1998) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). An ADI "not specified" was established at the 31st JECFA in 1987.

SYNONYMS Modified polydextroses; INS No. 1200

DEFINITION Randomly bonded condensation polymers of glucose with some sorbitol end-groups, and with citric acid or phosphoric acid residues attached to the polymers by mono or diester bonds. They are obtained by melting and condensation of the ingredients which consist of approximately 90 parts D-glucose, 10 parts sorbitol and up to 1 part citric acid or 0.1 part phosphoric acid. The 1,6-glucosidic linkage predominates in the polymers but other linkages are present. The products contain small quantities of free glucose, sorbitol, levoglucosan (1,6-anhydro-D-glucose) and citric acid and may be neutralized with any food-grade base and/or decolourized and deionized for further purification. The products may also be partially hydrogenated with Raney nickel catalyst to reduce residual glucose. Polydextrose-N is neutralized Polydextrose.

C.A.S. number 68424-04-4

Assay Not less than 90.0% of polymer on the ash-free and water-free bases

DESCRIPTION White to light tan-coloured solid. Polydextroses dissolve in water to give clear, colourless to straw-coloured solutions

FUNCTIONAL USES Bulking agent, humectant, stabilizer, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water

Test for sugar To 1 drop of 1 in 10 solution of the sample, add 4 drops of 5% phenol solution, then rapidly add 15 drops of sulfuric acid TS. A deep yellow to orange colour is produced.

Solubility in acetone With vigorous swirling add 1 ml of acetone to 1 ml of a 1 in 10 solution of the sample. The solution remains clear. With vigorous swirling add 2 ml of acetone to the solution. A heavy, milky turbidity develops immediately.

Test for reducing sugar To 1 ml of a 1 in 50 solution of the sample, add 4 ml of alkaline cupric citrate TS. Boil vigorously 2-4 min. Remove from heat and let precipitate (if any) settle. The supernatant is blue or blue-green.

PURITY

Water (Vol. 4) Not more than 4.0% (Karl Fischer Method)

pH (Vol. 4)	2.5 - 7.0 (for Polydextrose) (1 in 10 soln) 5.0 - 6.0 (for Polydextrose-N) (1 in 10 soln)
Sulfated ash (Vol. 4)	Not more than 0.3% (for Polydextrose) Not more than 2.0% (for Polydextrose-N)
Nickel (Vol. 4)	Not more than 2 mg/kg for hydrogenated polydextroses Use method *Nickel* for polyols
1,6-Anhydro-D-glucose	Not more than 4.0% on the ash-free and the dried bases See description under TESTS
Glucose and sorbitol	Not more than 6.0% combined on the ash-free and the dried bases; glucose and sorbitol are determined separately See description under TESTS
Molecular weight limit	Negative to test for polymer of molecular weight greater than 22,000 See description under TESTS
5-Hydroxymethylfurfural	Not more than 0.1% in Polydextrose Not more than 0.05% in Polydextrose-N See description under TESTS
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.”

TESTS

PURITY TESTS

Lead (Vol. 4)

Apparatus:
Use a suitable atomic absorption spectrophotometer (Perkin-Elmer Model 6000, or equivalent), a graphite furnace containing a L'vov platform (Perkin-Elmer Model HGA-500, or equivalent), and an autosampler (Perkin-Elmer Model AS-40, or equivalent).
Use a lead hollow cathode lamp (lamp current of 10 mA), a slit width of 0.7 mm (set low), the wavelength set at 283.3 nm, and a deuterium arc lamp for background correction. Use argon as the carrier gas. (Note: For this test use reagent-grade chemicals with as low a lead content as practicable, as well as high-purity water and gases. Before use, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse thoroughly with high-purity water.)

Lead Nitrate Stock Solution:
Dissolve 159.8 mg of reagent-grade lead nitrate in 1000.0 ml of water. Prepare and store this solution in glass containers that are free from lead salts. Each ml of this solution contains the equivalent of 100 µg of lead ion.

Standard Lead Solution:

On the day of use, dilute 10.0 ml of Lead Nitrate Stock Solution with water to 100.0 ml, and mix. Each ml of Standard Lead Solution contains the equivalent of 10 µg of lead ion.

Standard Solutions:
Prepare a series of lead standard solutions serially diluted from the Standard Lead Solution. Into separate 100 ml volumetric flasks, pipet 0.2, 0.5, 1, 2 ml, and 5 ml, respectively, of Standard Lead Solution, dilute to volume with water, and mix. The Standard Solutions contain, respectively, 0.02, 0.05, 0.1, 0.2, and 0.5 µg of lead per ml.

Matrix Modifier:
Transfer 100.0 mg of ammonium phosphate, dibasic ($(NH_4)_2HPO_4$) to a 10 ml volumetric flask, dilute to volume with water, and mix.
Sample Solution: Transfer about 1 g of the sample, accurately weighed, to a 10 ml volumetric flask, add 5 ml of water, and mix. Dilute to volume with water, and mix.

Spiked (fortified) Sample Solution:
Prepare a solution as directed under Sample Solution, but add 100 µl of the Standard Lead Solution, dilute to volume with water, and mix. This solution contains 0.1 µg/ml of added lead.

Procedure:
With the use of an autosampler, atomize 10-µl aliquots of the five Standard Solutions, using the following sequence of conditions: step (1) dry at 130° with a 20-sec ramp period, a 40-sec hold time, and a 300 ml/min argon flow rate; step (2) char at 800° with a 20-sec ramp period, a 40-sec hold time, and a 300 ml/min argon flow rate; step (3) atomize at 2400° for 6 sec with a 50 ml/min argon flow rate, and read; step (4) clean at 2600° with a 1-sec ramp period, a 5-sec hold time, and a 300 ml/min argon flow rate; and step (5) recharge at 20° with a 2-sec ramp period, a 20-sec hold time, and a 300 ml/min argon flow rate. Atomize 10 µl of the Matrix Modifier in combination with 10 µl of the Sample Solution under identical conditions used for the Standard Solutions. Repeat with 10 µl of the Matrix Modifier in combination with 10 µl of the Spiked Sample Solution.

Plot a standard curve using the concentration, in µg/ml, of each Standard Solution versus its maximum absorbance value compensated for background correction, and draw the best straight line. From the Standard Curve, determine the concentrations C_S and C_A in µg/ml, of the Sample Solution and the Spiked Sample Solution, respectively. Calculate the quantity, in mg/kg, of lead in the sample by the formula:

$$\frac{10 \times C_S}{W}$$

where
W = the weight, in g, of the sample taken.
Calculate the recovery by the formula:

$$\frac{C_S - C_A}{0.1} \times 100$$

where
0.1 = the amount of lead, in µg/ml, added to the Spiked Sample Solution.

1,6-Anhydro-D-glucose, glucose and sorbitol

Gas chromatography
- Octadecane Solution: Accurately weigh 50 mg of n-octadecane into a 100-ml volumetric flask and make up to volume with pyridine.
- Monomer Standard Solution: Weigh accurately 50 mg reagent grade alpha-D-glucose, 40 mg anhydrous D-sorbitol (min. 97% purity), and 35 mg of reagent grade (1,6-anhydro-D-glucose), into a 100-ml volumetric flask and make up to volume with pyridine.

Silylation of Monomer Standard Solution
Transfer 1.0 ml of Monomer Standard Solution to a screw-cap vial and add 1 ml of Octadecane Solution and 0.5 ml of N-trimethylsilylimidazole. Cap the vial and immerse in an ultrasonic bath at 70° for 60 min.

Gas Chromatograph conditions
Glass column, 2.44 m by 2 mm i.d. packed with 3% OV-1 on Gas Chrom Q 100/120 mesh. Flame ionization detector. Temperatures: column 175°; injection port 210°; detector 230°. Retention times (min): 1,6-anhydro-D-glucose, pyranose form 3.7; 1,6-anhydro-D-glucose furanose form (not present in standard) 4.3; n-octadecane 5.1; alpha-D-glucose 8.7; D-sorbitol 11.3; beta-D-glucose 13.3.

Procedure
Accurately weigh 20 mg of the sample into a screw-cap vial and add 1.0 ml of Octadecane Solution, 1 ml of pyridine, and 0.5 ml of N-trimethylsilylimidazole. Cap the vial and immerse in an ultrasonic bath at 70° for 60 min. Prior to sample analysis, inject 3 µl of the silylated Monomer Standard Solution into the gas chromatograph. Repeat two times, then inject 3 µl of the sample solution. Calculate the percentage of each monomer by the formula:

$$\frac{R \times W_S}{R_S \times W}$$

where
W = the weight of the sample in mg, adjusted for ash and moisture
W_S = the weight in mg of the monomer in the Monomer Standard Solution
R = the ratio of the area of the monomer peak to the area of the octadecane peak in the sample injection
R_S = the mean ratio of the area of the monomer peak to the area of the octadecane peak in the standard injections.
In the case of glucose, the peak areas for the alpha- and beta-epimers and in the case of 1,6-anhydro-D-glucose the peak areas for the pyranose form and furanose form are combined.

Molecular weight limit

Apparatus
Use a suitable high-pressure liquid chromatograph (HPLC) equipped with a differential refractometer, either a loop injector or suitable autosampler,

a column heating block or oven and a computing integrator, or computer data handling system with molecular weight determination capabilities. Use a Waters Ultrahydrogel 250 A size exclusion column, or equivalent. The column is maintained at 45^{o}, and the HPLC pump supplies eluent to it at 0.8 ml/min reproducible to 0.5%. The differential refractometer should be set at a sensitivity of 4×10^{-6} refractive index units full scale, and the plotter of the integrator should be set to 64 millivolts full scale. Maintain the detector cell at 35 ± 0.1^{o}. Noise attributable to the detector and electronics should be less than 0.1% full scale.

Eluent

The eluent is 0.1 N sodium nitrate containing 0.025% sodium azide. Dissolve 35.0 g of sodium nitrate and 1.0 g of sodium azide in 100 ml of HPLC-grade water. Filter through a 0.45-µm filter into a 4-l flask. Dilute to volume with HPLC-grade water. De-gas by applying an aspirator vacuum for 30 min.

Standard Solution

Transfer 20 mg each of dextrose, stachyose, 5800, 23,700, and 100,000 MW pullulan standards into a 10-ml volumetric flask. Dissolve in and dilute to volume with Eluent. Filter through a 0.45 µm syringe filter into a suitable autosampler vial, and seal (All components of the Standard Solution are available from Polymer Laboratories, Inc., Technical Center, Amherst Fields Research Park, 160 Old Farm Road, Amherst, MA 01002, USA).

Column Equilibration

After installation of a new column in the HPLC, pump Eluent through it overnight at 0.3 ml/min. Before calibration or analysis, increase the flow slowly to 0.8 ml/min over a 1-min period, then pump at 0.8 ml/min for at least 1 h before the first injection. Check the flow gravimetrically, and adjust it if necessary. Reduce the flow to 0.1 ml/min when the system is not in use.

Data System Setup

Set the integrator or computerized data handling system as their respective manuals instruct for normal gel permeation chromatographic determinations. Set the integration time to 15 min.

Column Standardization

After the HPLC system has been equilibrated at a flow rate of 0.8 ml/min for at least 1 h, inject 50 µl of the Standard Solution five times, allowing 15 min between injections. Record the retention times of the various components in the Standard Solution. Retention times for each component should agree within ± 2 sec. Insert the average retention time along with the molecular weight of each component into the calibration table of the molecular weight distribution software.

System Suitability

Check the regression results for a cubic fit of the calibration points. They should have an R^2 value of 0.9999+. Dextrose and stachyose should be baseline-resolved from one another and from the 5800 MW pullulan standard. Elevated valleys are usually observed between the 5800,

23,700 and 100,000 MW pullulan standards.

Sample Preparation
Transfer 50 mg of sample, accurately weighed, into a 10-ml volumetric flask. Dissolve in and dilute to volume with Eluent. Filter through a 0.45-µm syringe filter into a suitable autosampler vial.

Procedure
Inject 50 µl of the Sample Preparation, following the same conditions and procedure as described under Column Standardization. Using the Formula weight Distribution software of the data reduction system, generate a molecular weight distribution curve of the sample. There is no measurable peak above a molecular weight of 22,000.

5-Hydroxymethylfurfural (HMF)

Principle
HMF solutions absorb light in the ultraviolet region. The maximum absorption occurs at 283 nm and the molar extinction coefficient is 16,830 at that wavelength. The HMF concentration in polydextrose solutions is determined from the optical density at 283 nm and the application of the Beer-Lambert law.

Apparatus
- Standard laboratory equipment
- Ultraviolet spectrophotometer
- Spectrophotometer cells (quartz), 1.00 cm path length

Procedure
Accurately weigh 1.00±0.01 g of the sample into a 100-ml volumetric flask and make up to volume with distilled water (for polydextrose-N 70% solution use 1.43 ± 0.01 g sample). Read the optical density of this solution against a water blank at 283 nm in a 1.00 cm quartz cell in the spectrophotometer according to the directions supplied with the instrument. Under these conditions, the % HMF in the original sample is 0.0749 x optical density, on the dried basis.

Calculation

$$C = \frac{100 \times M \times D}{10 \times L \times E}$$

where
C = % HMF in the original polydextrose sample
M = HMF molecular weight
D = optical density of the solution
L = the path length of the spectrophotometer cell
E = the molar extinction coefficient for HMF
The numbers 100 and 10 are factors to convert solution concentration in mg/l to % HMF in the original sample, on the dried basis.

METHOD OF ASSAY

Phenol Solution
Add 20 ml of water to 80 g of phenol.

Glucose Standard Solutions

Weigh accurately 100 mg of alpha-D-glucose (minimum 97% purity) into a 500-ml volumetric flask and make up to volume with distilled water. Dilute five aliquots of the solution with distilled water to obtain the following concentrations of standard: 50, 40, 20, 10 and 5 µg/ml.

Standard Curve

Run each analysis in triplicate. On a daily basis, pipet 2.0 ml of each of the Glucose Standard Solutions into 4-dram (14.8 ml) acetone-free screw-cap vials. Add 0.12 ml of the phenol solution and mix gently. Uncap vials and add rapidly 5 ml of sulfuric acid TS. Immediately recap the vials and shake vigorously.

CAUTION: Rubber gloves and safety shield should be used in the sulfuric acid addition step.

Let the vials stand at room temperature for 45 min. Determine absorbances at 490 nm in a suitable spectrophotometer, using a Phenol Solution-sulfuric acid mixture as a blank in the reference cell. Plot mean absorbances versus concentrations in µg/ml.

Procedure

In triplicate, weigh accurately about 250 mg of the sample into a 250-ml volumetric flask and make up to volume with distilled water. Transfer a 10 ml aliquot to a 250-ml volumetric flask and make up to volume with distilled water. Proceed as in Standard Curve. Calculate the percentage of polymer by the formula:

$$\text{Polymer (\%)} = 1.05 \times \frac{100 \times A}{S \times C} - P_G - 1.11 \times P_1$$

where

A = the sample absorbance

S = the slope of absorbance versus glucose concentration in µg/ml obtained from the Standard Curve

C = the concentration of the sample solution in µg/ml (adjusted for ash and moisture)

P_G and P_1 = the percentages of glucose and 1,6-anhydro-D-glucose, respectively, determined by the tests for monomers (see Purity tests for 1,6-Anhydro-D-glucose, glucose and sorbitol as described above).