α-CYCLODEXTRIN

New specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001). An ADI "not specified" was established at the 57th JECFA (2001).

SYNONYMS α-Schardinger dextrin, α-dextrin, cyclohexaamylose, cyclomaltohexaose, α-cycloamylase

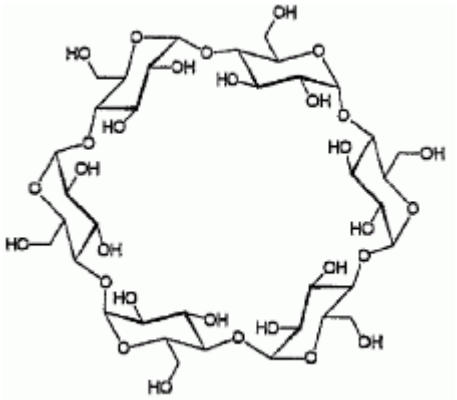
DEFINITION A non-reducing cyclic saccharide consisting of six α-1,4-linked D-glucopyranosyl units produced by the action of cyclodextrin glucosyltransferase (CGTase, EC 2.4.1.19) on hydrolyzed starch. Recovery and purification of α-cyclodextrin may be carried out using one of the following procedures: precipitation of a complex of α-cyclodextrin with 1-decanol, dissolution in water at elevated temperature and reprecipitation, steam-stripping of the complexant, and crystallization of α-cyclodextrin from the solution; or chromatography with ion-exchange or gel filtration followed by crystallization of α-cyclodextrin from the purified mother liquor; or membrane separation methods such as ultra-filtration and reverse osmosis.

Chemical names: Cyclohexaamylose

C.A.S. number: 10016-20-3

Chemical formula: $(C_6H_{10}O_5)_6$

Structural formula:



Formula weight: 972.85

Assay: Not less than 98% (dry basis)

DESCRIPTION Virtually odourless, white or almost white crystalline solid

FUNCTIONAL USES Carrier; encapsulation agent for food additives, flavourings, and vitamins; stabilizer; absorbent

CHARACTERISTICS

IDENTIFICATION

Melting range (Vol. 4): Decomposes above 278 °

Solubility (Vol. 4)	Freely soluble in water; very slightly soluble in ethanol
Specific rotation (Vol. 4)	[α]25D: Between +145° and +151° (1% solution)
Chromatography	The retention time for the major peak in a liquid chromatogram of the sample corresponds to that for α-cyclodextrin in a chromatogram of reference α-cyclodextrin (available from Consortium für Elektrochemische Industrie GmbH, München, Germany or Wacker Biochem Group, Adrian, MI, USA) using the conditions described in the METHOD OF ASSAY.
PURITY	
Water (Vol. 4)	Not more than 11% (Karl Fischer Method)
Residual complexant (1-decanol)	Not more than 20 mg/kg See description under TESTS
Reducing substances	Not more than 0.5% (as dextrose) See description under TESTS
Sulfated ash (Vol. 4)	Not more than 0.1%
Lead (Vol. 4)	Not more than 1 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

Residual complexant (1-decanol)

Principle
After enzymatic digestion of the sample followed by solid-phase extraction, 1-decanol is determined by gas chromatography.

Buffer solution
Dissolve 606 mg Tris-buffer (Sigma-Aldrich, St. Louis, MO, USA) and 430 mg calcium sulfate (dihydrate) in 500 ml of water. Adjust pH with concentrated phosphoric acid to pH 6.5.

Internal standard (IS) solution
Add 50 mg 1-octanol (chromatography grade) to 250 ml tetrahydrofuran (THF).

Reference solution (25 mg/kg 1-decanol)
Dissolve 75.0 mg 1-decanol (chromatography grade) in 100 ml IS solution. Transfer 200 :l of this stock solution into a 20-ml volumetric flask and fill with IS solution to the mark.

Sample solution

Dissolve 750 mg of sample and 50 mg of glucoamylase, EC 3.2.1.3, (e.g.,Gluczyme 8000, available from Wacker Chemie, Munich, Germany) in 7 ml of a 10 mM Tris-buffer (pH 6.5). Add 100 :l IS solution and 50 :l cyclodextrin glucosyltransferase preparation (500 U/ml) (Wacker Chemie, Munich, Germany). Close tightly, mix and incubate in a shaking water bath at 40° for 4 hours.

Condition an extraction column (Isolute C18 (10 ml) - ICT, Bad Homburg, Germany - or similar) by washing with methanol (2x10 ml) and water (4x10 ml). Pass the incubated solution slowly through the column and wash with water (2x10 ml). Gently pass nitrogen gas through the column to dry it (10 min). Apply 2.5 ml of THF to the column and let stand for 5 minutes. Then, elute the sample solution slowly.

Gas chromatography
Column - Hewlett Packard HP-1 (25 m x 0.32 mm), 0.5:m FD
Carrier gas - helium (flow rate: 1 ml/min)
Detector - flame ionization
Temperatures - injection port: 265°; column: initial 60° (1 min isotherm); heating rate 20°/min; final 300° (7 min isotherm)
Injection volume: 1 µl
Measure the areas of the 1-decanol and 1-octanol peaks in the reference solution and sample solution.

Calculation
The concentration of 1-decanol (mg/kg) in the sample is:
$C_{dec} = (C_{dec})_R \times (A_{dec}/A_{oct})_S / (A_{dec}/A_{oct})_R$
where $(C_{dec})_R$ is the concentration of 1-decanol (mg/kg) of the reference solution; and $(A_{dec}/A_{oct})_S$ and $(A_{dec}/A_{oct})_R$ are the peak area ratios for 1-decanol to 1-octanol in the sample solution and reference solution, respectively.

Reducing substances

Note
Reducing substances are determined as dextrose. Dextrose levels are usually lower when determined by the following procedure in the presence of α-cyclodextrin compared to levels determined in its absence. Therefore, α-cyclodextrin reference standard is included in the procedure for the construction of the calibration curve.

Reagent solution
Weigh 10.0 g 3,5-dinitrosalicylic acid in a 1000-ml volumetric flask. Add 80 ml water and dissolve the 3,5-dinitrosalicylic acid by heating in a water bath. Prepare a solution of 16.0 g sodium hydroxide in 200 ml water and a solution of 300 g sodium potassium tartrate in 500 ml water. Transfer both solutions to the 1000-ml flask. Fill with water to the mark, shake the flask and let it stand for 24 hours. Filter (paper) the reagent solution prior to use if a precipitate appears.
Reference solution
Weigh accurately 1.0 g dextrose (anhydrous) in a 100-ml volumetric flask and fill with water to the mark.

Test solution
Weigh accurately 10.0 g of test sample into a 100-ml volumetric flask, add

80-90 ml water and dissolve the test sample (ultra-sonification bath, 15 minutes, 30°). Fill with water to the mark.

Calibration solutions
Weigh 1.0 g of reference α-cyclodextrin (available from Consortium für Elektrochemische Industrie GmbH, München, Germany or Wacker Biochem Group, Adrian, MI, USA) into each of eleven 10-ml volumetric flasks (numbered 0 to 10). Add 0, 0.1, 0.2, ..., 1.0 ml of reference solution to flasks nos. 0, 1, ... to 10, respectively. Fill all flasks with water to the mark.

Calibration curve
Assemble a set of eleven 10-ml volumetric flasks. Transfer 1 ml of each of the eleven calibration solutions into the flasks and add 1 ml of reagent solution to each flask. Heat each flask in the boiling water bath for 10 minutes. Cool rapidly to room temperature and fill with water to the mark. For each solution, measure the absorbance against water at 545 nm. Plot the data as absorbance vs. dextrose concentration (mg/ml).

Analysis
Prepare a set of six 10-ml volumetric flasks (labeled a through f) and add 1 ml of the reagent solution to each. Transfer 1 ml of the test solution to flasks a, b, and c. Transfer 1 ml of the calibration solutions numbered 0, 3, and 6 to flasks d, e, and f. Thoroughly mix the contents of each flask and place in a boiling water bath for 10 minutes. Then, cool the flasks to room temperature, fill to the mark with water, and measure absorbance of the solutions against water at 545 nm.

Evaluation and calculation
The result is only valid if the absorbances of the solutions in flasks d-f do not deviate more than 5% from the calibration curve. Determine the reducing substance concentrations (mg/ml) of the solutions in flasks a-c and calculate their mean, $C_{RS.}$
Then,
$W_{RS} = 10\ C_{RS}$
And
% reducing substances = $100 \times W_{RS}/0.01W_{TS}$
where W_{RS} is the mean weight (mg) of reducing substance (as dextrose), determined from the absorbance readings, and W_{TS} is the weight (mg) of the test sample used to prepare the test solution.

METHOD OF ASSAY

Determine by liquid chromatography (see Volume 4) using the following conditions:

Sample solution
Weigh accurately about 100 mg of test sample into a 10-ml volumetric flask and add 8 ml of deionized water. Dissolve the sample completely using an ultra-sonification bath (10-15 min) and dilute to the mark with purified deionized water. Filter through a 0.45-micrometer filter.

Reference solution
Weigh accurately about 100 mg of reference α-cyclodextrin into a 10-ml volumetric flask and add 8 ml of deionized water. Dissolve the sample

completely using an ultra-sonification bath (10-15 min) and dilute to the mark with purified deionized water.

Chromatography

Liquid chromatograph equipped with a refractive index detector and an integrating recorder.
Column and packing: Nucleosil-100-NH2 (10 µm) (Machery & Nagel Co., Düren, Germany) or similar
- length: 250 mm
- diameter: 4 mm
- temperature: 40°
Mobile phase: acetonitrile/water (67/33, v/v)
Flow rate: 2.0 ml/min
Injection volume: 10 µl

Procedure

Inject the sample solution into the chromatograph, record the chromatogram, and measure the area of the α-cyclodextrin peak. Repeat for the reference solution. Calculate the percentage of α-cyclodextrin in the test sample as follows:

$$\% \text{ α-cyclodextrin (dry basis)} = 100 \times (A_S/A_R)(W_R/W_S)$$

where:
A_S and A_R are the areas of the peaks due to α-cyclodextrin for the sample solution and reference solution, respectively.
W_S and W_R are the weights (mg) of the test sample and reference α-cyclodextrin, respectively, after correcting for water content.