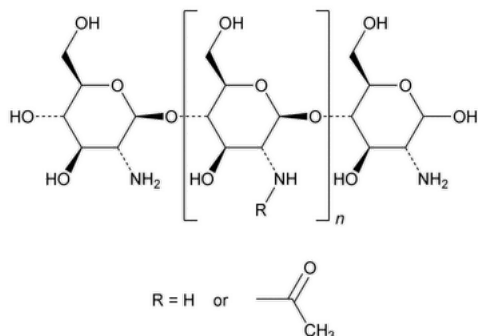


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Chitosan



Poly- β -(1,4)-2-amino-2-deoxy-D-glucose

CAS RN[®]: 9012-76-4.

DEFINITION

Chitosan is an unbranched binary polysaccharide consisting of *N*-acetyl-D-glucosamine and D-glucosamine units linked in a β (1→4) manner.

Chitosan is obtained by partial deacetylation of chitin, which is extracted from the shells of edible shrimps and crabs suitable for human use.

Its degree of deacetylation is NLT 70.0% and NMT 95.0%.

IDENTIFICATION

Change to read:

• **A.** **SPECTROSCOPIC IDENTIFICATION TESTS (197), Infrared Spectroscopy: 197A** (CN 1-MAY-2020)

• **B.**

Sample: 0.2 g of Chitosan powder

Analysis: Add 80 g of water to the *Sample*, and stir briefly to obtain a dispersion. Separately prepare a glycolic acid solution by dissolving 0.1 g of glycolic acid in 20 g of water. Add the solution in one step to the dispersion. Stir the mixture gently at room temperature until it becomes a clear solution. [NOTE—It takes approximately 30–60 min to obtain a clear solution.] Add 5 g of a 0.5% sodium lauryl sulfate aqueous solution to the clear solution.

Acceptance criteria: A gelatinous mass is formed.

ASSAY

Change to read:

• **DEGREE OF DEACETYLATION**

[NOTE—If tetramethylsilane is not used as the NMR reference, a suitable signal of the solvent itself can be used as a reference.]

Solvent: Deuterated formic acid

NMR reference: Tetramethylsilane

Sample solution: Into a 20-mL scintillation vial with a screw cap, dissolve 5–10 mg of Chitosan in deuterated formic acid, containing 0.5%–1.0% of tetramethylsilane, to obtain 1 mL of solution. Tightly close the vial, and dissolve Chitosan using a magnetic stirrer. To completely dissolve takes it about 48 h; the stirring is stopped when a clear solution with a high viscosity is obtained. Break up any clumps formed during the dissolution process with a spatula, if necessary.

Analysis

Sample: *Sample solution*

Transfer 0.5–1.0 mL of the *Sample solution* to a standard 5-mm NMR spinning tube. Proceed as directed in [Nuclear Magnetic Resonance Spectroscopy \(761\), Relative Method of Quantitation](#), scanning the region from 0–7 ppm, and using the calculation formulas below.

Record as A_1 the average area of the composite band from about 6–3 ppm, representing the seven protons with oxygen neighbors in the sugar ring. Record as A_2 the average area of the signals at about 2 ppm, due to the methyl groups of the acetyl units, with reference to the tetramethylsilane singlet at 0 ppm.

Calculate the percentage of deacetylation degree, by weight, in the Chitosan taken:

$$\text{Result} = \{1 - [(7 \times A_2) / (3 \times A_1)]^{\Delta}\} \times 100 \quad (\text{ERR 1-JUL-2019})$$

Acceptance criteria: The degree of deacetylation is NLT 70.0% and NMT 95.0%.

IMPURITIES

- [RESIDUE ON IGNITION \(281\)](#): NMT 1.0%
- **LIMIT OF LEAD, MERCURY, CHROMIUM, NICKEL, CADMIUM, AND ARSENIC**

65% Nitric acid: Use ultratrace nitric acid, 65%–70% HNO₃, ACS reagent grade.

Internal standard: Transfer 0.2 mL of a solution containing 1000 ppm of yttrium [NOTE—Yttrium ICP standard solutions are commercially available.¹] and 0.2 mL of a solution containing 1000 ppm of lutetium [NOTE—Lutetium ICP standard solutions are commercially available.²] to a 100-mL volumetric flask, add 1 mL of 65% Nitric acid, dilute with water to volume, and mix.

Blank standard: Transfer 1.0 mL of the Internal standard to a 100-mL volumetric flask, add 1 mL of 65% Nitric acid, dilute with water to volume, and mix.

Standard solutions: Transfer 0.1 mL of a solution containing 10 ppm of each of lead, mercury, chromium, nickel, cadmium, and arsenic [NOTE—Multi-element ICP standard solutions are commercially available.³] to a 100-mL volumetric flask, add 1 mL of the Internal standard and 1 mL of 65% Nitric acid, and dilute with water to volume (Standard solution 0.01 ppm). Transfer 0.1 mL of a solution containing 10 ppm of each of lead, mercury, chromium, nickel, cadmium, and arsenic to a 1000-mL volumetric flask, add 10 mL of the Internal standard and 10 mL of 65% Nitric acid, and dilute with water to volume (Standard solution 0.001 ppm).

Sample solution: Transfer 1.0 g of Chitosan to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and add a sufficient quantity of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to moisten the substance thoroughly. Warm gently until the reaction commences, allow the reaction to subside, and add portions of the same acid mixture, heating after each addition, until a total of 18 mL of the acid mixture has been added. Increase the amount of heat, and boil gently until the solution darkens. Cool, add 2 mL of nitric acid, and heat again until the solution darkens. Continue the heating, followed by addition of nitric acid until no further darkening occurs, then heat strongly to the production of dense, white fumes. Cool, cautiously add 5 mL of water, boil gently to the production of dense, white fumes, and continue heating until the volume is reduced to a few mL. Cool, cautiously add 5 mL of water, and examine the color of the solution. If the color is yellow, cautiously add 1 mL of 30% hydrogen peroxide, and again evaporate to the production of dense, white fumes and a volume of 2–3 mL. If the solution is still yellow, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, rinse into a 20-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of digestion solution to a 100-mL volumetric flask, and retain the remaining digestion solution for use in the Limit of Iron. Add 1 mL of the Internal standard to the 100-mL volumetric flask, dilute with water to volume, and mix.

Blank solution: Prepare the blank digestion solution, following the preparation procedure for the Sample solution, but without using Chitosan. Transfer 5.0 mL of blank digestion solution to a 100-mL volumetric flask, and retain the remaining digestion solution for use in the Limit of Iron. Add 1 mL of the Internal standard to the 100-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions

(See [Plasma Spectrochemistry \(730\)](#).)

Mode: Inductively coupled plasma–mass spectrometer (ICP–MS)

Spectrometer: Quadrupole mass spectrometer

Detector: Ion detector maintained under vacuum

Other requirements: The instrument should read all isotopes for the following elements shown in [Table 1](#), for the yttrium internal standard (89 amu) and the lutetium internal standard (175 and 176 amu), and should report the total element contents using the most naturally abundant isotopes.

Table 1

Element	Isotope (amu)
Lead	208
	209
Mercury	201
	202
Chromium	53
Nickel	58
	60
Cadmium	114
Arsenic	75

Analysis

Samples: Blank standard, Standard solutions, Sample solution, and Blank solution

Instrument performance must be verified to conform to the manufacturer’s specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Perform the evaluation using instrument software such as

correction equations for interferences and taking the *Internal standard* into account. Generate the calibration curve using the *Blank standard*, *Standard solution 0.001 ppm*, and *Standard solution 0.01 ppm*: the linear regression coefficient is NLT 0.999.

Aspirate the *Blank solution* and *Sample solution*, respectively, at least in duplicate, and report the average reading as each element content of the sample. Determine the concentration C_B , in $\mu\text{g/mL}$, of each element in the *Blank solution*, and also determine the concentration C_S , in $\mu\text{g/mL}$, of each element in the *Sample solution* using the calibration curve.

Calculate the quantity, in $\mu\text{g/g}$, of each element in the portion of Chitosan taken:

$$\text{Result} = [(C_S - C_B)/W] \times F \times V$$

F = dilution factor, 4

V = volume of the *Sample solution*, 100 mL

W = weight of the Chitosan taken to prepare the *Sample solution* (g)

Acceptance criteria: See [Table 2](#).

Table 2

Element	Acceptance Criteria (ppm)
Lead	NMT 0.5
Mercury	NMT 0.2
Chromium	NMT 1.0
Nickel	NMT 1.0
Cadmium	NMT 0.2
Arsenic	NMT 0.5

• **LIMIT OF IRON**

65% Nitric acid: Use ultratrace nitric acid, 65%–70% HNO_3 , ACS reagent grade.

Blank standard: Prepared as directed in the *Limit of Lead, Mercury, Chromium, Nickel, Cadmium, and Arsenic*.

Standard stock solution 100 ppm: Immediately before use, dilute an appropriate amount of iron standard⁴ with a solution of 65% *Nitric acid* (1 in 100) to prepare an acidic solution containing the equivalent of 100 $\mu\text{g/mL}$ of iron.

Standard solutions: Separately transfer 0.1 and 0.5 mL of *Standard stock solution 100 ppm* to 100-mL volumetric flasks, dilute with a solution of 65% *Nitric acid* (1 in 100) to volume, and mix. These solutions contain, respectively, 0.1 and 0.5 μg of iron/mL (*Standard solution 0.1 ppm* and *Standard solution 0.5 ppm*).

Sample solution: Transfer 10.0 mL of the digestion solution from the test for *Limit of Lead, Mercury, Chromium, Nickel, Cadmium, and Arsenic* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Blank solution: Transfer 10.0 mL of the blank digestion solution from the test for *Limit of Lead, Mercury, Chromium, Nickel, Cadmium, and Arsenic* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Instrumental conditions

(See [Plasma Spectrochemistry \(730\)](#).)

Mode: Inductively coupled plasma–atomic emission spectrometer (ICP–AES)

Analytical wavelength: 238.040 and 239.562 nm with the settings optimized as directed by the manufacturer

Analysis

Samples: *Blank standard*, *Standard solutions*, *Sample solution*, and *Blank solution*

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the *Blank standard*, *Standard solution 0.1 ppm*, and *Standard solution 0.5 ppm*: the linear regression coefficient is NLT 0.999.

Aspirate the *Blank solution* and *Sample solution*, respectively, at least in duplicate, and report the average reading as the iron content of the sample. Determine the concentration C_B , in $\mu\text{g/mL}$, of iron in the *Blank solution*, and also determine the concentration C_S , in $\mu\text{g/mL}$, of iron in the *Sample solution* using the calibration curve.

Calculate the quantity, in $\mu\text{g/g}$, of iron in the portion of Chitosan taken:

$$\text{Result} = [(C_S - C_B)/W] \times F \times V$$

F = dilution factor, 2

V = volume of the *Sample solution*, 100 mL