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Dextran 70

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click https://www.uspnf.com/rb-dextran-70-20220325.

Dextrans.

DEFINITION

Dextran 70 is derived by controlled hydrolysis and fractionation of polysaccharides elaborated by the fermentative action of certain appropriate strains of *Leuconostoc mesenteroides* (NRRL, B-512F; NCTC, 10817) on a sucrose substrate. It is a glucose polymer in which the linkages between glucose units are almost entirely of the α-1:6 type. Its weight average molecular weight is in the 63,000–77,000 range.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K

• **B.**

Sample solutions: Prepare four solutions of Dextran 70 in water in such a manner that the concentrations are accurately known and approximately evenly distributed in the range of 2%–0.5%.

Analysis: Using a capillary tube viscometer having dimensions such that the flow time of water is NLT 100 s, measure the flow times of water and of the *Sample solution* at 20°.

Calculate the viscosity numbers of each of the *Sample solutions*:

$$\text{Result} = \{\ln[(R_D) \times (t/t_0)]\}/C$$

R_D = ratio of the density of the individual *Sample solution* to that of water

t = *Sample solution* flow time

t_0 = water flow time

C = concentration of Dextran 70 in the *Sample solution* (g/mL)

Plot the viscosity numbers of each of the *Sample solutions* against their respective concentrations, draw the straight line of best fit through the points, and extrapolate to zero concentration.

Acceptance criteria: The value of the intercept is 24–29 mL/g.

IMPURITIES

• **Chloride and Sulfate, *Sulfate* ⟨221⟩:** A 1.5-g portion of Dextran 70 shows NMT 0.03%, which corresponds to 0.45 mL of 0.020 N sulfuric acid.

• **Limit of Nitrogenous Impurities** (where it is labeled as intended for use in the preparation of injectables)

Sulfate solution: To 1000 mL of sulfuric acid add 5 g of anhydrous cupric sulfate and 500 g of potassium sulfate. Dissolve by heating, and store at 60°. [Note—If storage at 60° is not possible, prepare a smaller quantity of *Sulfate solution* on the day of use, adjusting the proportions accordingly.]

Indicator: Dilute a mixture of 20 mL of a 0.1% solution of bromocresol green in alcohol and 4 mL of methyl red TS with water to 100 mL.

Sample solution: Transfer 0.2 g of Dextran 70, accurately weighed, to a micro-Kjeldahl flask. Add 4 mL of *Sulfate solution*.

Analysis

Sample: *Sample solution*

Heat until the solution exhibits a clear green color and the sides of the flask are free from carbonaceous material. Cool, and transfer the solution to a steam distillation unit. Rinse the Kjeldahl flask three times with 5 mL of water, adding the washings to the solution. Add 15 mL of 45% sodium hydroxide solution, immediately close the distillation apparatus, and commence steam distillation without delay. Receive the distillate in 1 mL of *Indicator* in a 100-mL flask, keeping the end of the condensing tube below the liquid surface for 5 min and above the liquid surface for 1 min. Upon completing the distillation, remove the receiving flask, and rinse the end of the condensing tube with a small quantity of water, adding the rinse to the distillate. Titrate the distillate with 0.010 N hydrochloric acid until the color changes from blue to reddish violet. Perform a blank determination, and make any necessary correction.

Acceptance criteria: The corrected volume of 0.010 N hydrochloric acid titrated does not exceed 0.14 mL (0.01%, as N).

• **Limit of Alcohol and Related Impurities**

Standard solution: To 25.0 mL of the *Sample solution* add 0.5 mL of a 2.5% (w/v) solution of *n*-propyl alcohol.

Sample solution: Dissolve without heating 5.0 g of Dextran 70 in 100 mL of water, and distill the solution, collecting the first 45 mL of the distillate. Dilute the distillate with water to 50.0 mL, and mix.

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Chromatographic system

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m; packed with S3

Temperatures

Column: 160°

Injection port: 240°

Detector: 210°

Carrier gas: Nitrogen

Flow rate: 25 mL/min

[Note—Injector seals may deteriorate after multiple injections of the *Standard solution* and *Sample solution*. Inspect the seals before making a series of injections.]

Injection volume: 1 µL

Analysis

Samples: *Standard solution*, *Sample solution*, and a 0.05% (w/v) solution of *n*-propyl alcohol and water

Acceptance criteria: After corrections for any impurities in the *n*-propyl alcohol solution and water, the total area of peaks from impurities in the *Sample solution* does not exceed the area of the *n*-propyl alcohol solution peak.

• **Antigenic Impurities** (where it is labeled as intended for use in the preparation of injectables)

Sample solution: Prepare a sterile solution containing 60 mg/mL of Dextran 70 in sodium chloride injection.

Analysis: At intervals of about 48 h, inject three 0.5-mL doses into the peritoneal cavities of each of six guinea pigs. At 14 days after the first intraperitoneal injection, inject 0.20 mL intravenously into each of three of the guinea pigs, and at 21 days treat the other three guinea pigs similarly. Observe the animals for 30 min after each intravenous injection and again 24 h later.

Acceptance criteria: The animals exhibit no evidence of anaphylactoid reactions, such as coughing, bristling of hair, or respiratory distress.

SPECIFIC TESTS

• **Optical Rotation,** *Specific Rotation* ⟨781S⟩

Sample solution: 20 mg/mL of Dextran 70, heated, if necessary, on a water bath to dissolve

Acceptance criteria: +195° to +203°

• **pH** ⟨791⟩

Sample solution: A solution (6 in 100)

Acceptance criteria: 4.5–7.0

• **Loss on Drying** ⟨731⟩

Analysis: Dry at 105° for 5 h.

Acceptance criteria: NMT 7.0%

• **Bacterial Endotoxins Test** ⟨85⟩(where it is labeled as intended for use in the preparation of injectables)**:** When tested in sodium chloride injection (0.6 in 10), it contains NMT 0.5 USP Endotoxin Unit/mL.

• **Color of Solution**

Sample solution: A solution in water (6 in 100)

Blank solution: Water

Analysis: Absorbance at 375 nm in a 4-cm cell

Acceptance criteria: NMT 0.15

• **Safety:** Inject intravenously 1.0 mL of a sterile solution of 6% Dextran 70 in saline TS into each of five mice weighing 18–20 g. The injection period is 10–15 s. If there are no deaths within 72 h, it meets the requirements of the test. If one or more animals die, continue the test using 10 mice weighing 20 ± 0.5 g. If all animals survive for 72 h, the requirements of the test are met.

Change to read:

• **Molecular Weight Distribution** and **Weight and Number Average Molecular Weights**

Mobile phase: 7.1 g/L of anhydrous sodium sulfate in water, filtered and degassed

Calibration solutions: Separately prepare 20 mg/mL in *Mobile phase*, USP Dextran 4 Calibration RS, USP Dextran 10 Calibration RS, USP Dextran 40 Calibration RS, USP Dextran 70 Calibration RS, and USP Dextran 250 Calibration RS

Marker solution: 3 mg/mL each of dextrose and USP Dextran V0 Marker RS in *Mobile phase*

System suitability solution: 20 mg/mL of ▲USP Dextran 70 RS▲ (RB 1-Jul-2022) in *Mobile phase*

Sample solution: 20 mg/mL of Dextran 70 in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: Three 7.5-mm × 30-cm columns; packing L38

Column temperature: Constant temperature

Injection volume: 50 µL

System suitability

Samples: *Calibration solutions*, *Marker solution*, and *System suitability solution*

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Suitability requirements

Elution profile: Profile shows two peaks: the first due to the V_0 marker, the second due to dextrose, *Marker solution*

Tailing factor: NMT 1.3 for the dextrose peak, *Marker solution*

Relative standard deviation of the ratio V_0 :V_T : Determine the void volume, V_0, of the system as the inflection point of the ascending part of the first peak. Determine the total volume, V_T, of the system as the maximum of the second peak. The relative standard deviation of the ratio V_0:V_T is NMT 1%, *Marker solution*

Weight average molecular weight: Chromatograph each of the *Calibration solutions* separately. Divide each profile into at least 60 vertical sections of equal volume increments. (The actual number of sections is represented by the variable *a* in the equations below.) Record y_i, the height above the baseline, corresponding to each value of v_i, the volume eluted at that section. For each value of v_i, calculate the distribution coefficient, K_i:

$$\text{Result} = (v_i - V_0)/(V_T - V_0)$$

Find appropriate values of b_1, b_2, b_3, b_4, and b_5 using a suitable method[1] that, when substituted in the equation:

$$M_i = b_5 + e^{(b_4 + b_1 K_i + b_2 K_i^2 + b_3 K_i^3)}$$

and the resulting values of M_i substituted, along with their corresponding values of y_i, in the equation:

$$\overline{M}_W = \sum_{i=1}^{a}\left(y_i M_i\right) / \sum_{i=1}^{a} y_1$$

give values of weight average molecular weight, $\overline{M}_w$, within 5% of the labeled values for each of the *Calibration solutions* and 180 ± 2 for dextrose. Using the *System suitability solution*, calculate $\overline{M}_w$ of the total molecular weight distribution using the same method, but inserting the now known values of b_1, b_2, b_3, b_4, and b_5.

▲See the USP Dextran 70 RS certificate for the range.▲ (RB 1-Jul-2022)

Weight average molecular weight, high-fraction: Calculate $\overline{M}_w$ of the high-fraction dextran eluted through section *n*:

$$\sum_{i=1}^{n}(y_i M_i) / \sum_{i=1}^{n} y_i$$

in which *n* is defined by the relations:

$$\sum_{i=1}^{n} y_i \le 0.1\left(\sum_{i=1}^{a} y_i\right) \quad \text{and}$$

$$\sum_{i=1}^{n+1} y_i > 0.1\left(\sum_{i=1}^{a} y_i\right)$$

▲See the USP Dextran 70 RS certificate for the range.▲ (RB 1-Jul-2022)

Weight average molecular weight, low-fraction: Calculate $\overline{M}_w$ of the low-fraction dextran eluted in and after section *m*:

$$\sum_{i=m}^{a}(y_i M_i) / \sum_{i=m}^{a} y_i$$

in which *m* is defined by:

$$\sum_{i=m}^{a} y_i \le 0.1\left(\sum_{i=1}^{a} y_i\right) \quad \text{and}$$

$$\sum_{i=m-1}^{a} y_i > 0.1\left(\sum_{i=1}^{a} y_i\right)$$

▲See the USP Dextran 70 RS certificate for the range.▲ (RB 1-Jul-2022)

Analysis

Sample: *Sample solution*

Calculate the weight average molecular weight, $\overline{M}_w$, for the total molecular weight distribution, the high-fraction dextran, and the low-fraction dextran. With the values of b_1, b_2, b_3, b_4, and b_5 obtained with the *Calibration solutions* in *System suitability*, calculate the number average molecular weight, $\overline{M}_n$, of the total molecular weight distribution of the *Sample solution* by substituting the corresponding values of M_i, along with their corresponding values of y_i, in the equation:

$$\overline{M}_n = \sum_{i=1}^{a} y_i / \sum_{i=1}^{a}(y_i / M_i)$$

Acceptance criteria: The weight average molecular weight, $\overline{M}_w$, of the total molecular weight distribution is 63,000–77,000. The weight average molecular weights, $\overline{M}_w$, of the high-fraction dextran and of the low-fraction dextran are NMT 195,000 and NLT 13,000, respectively.

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The number average molecular weight, $\overline{M}_n$, is 34,000–48,000. Where Dextran 70 is labeled as intended for use in the preparation of injectables, the ratio $\overline{M}_w$:$\overline{M}_n$ is in the 1.4–1.9 range.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at 25°; excursions are permitted between 15° and 30°.

• **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

Change to read:

• **USP Reference Standards** ⟨11⟩

USP Dextran 70 RS
USP Dextran 4 Calibration RS
USP Dextran 10 Calibration RS
USP Dextran 40 Calibration RS
USP Dextran 70 Calibration RS
USP Dextran 250 Calibration RS

▲▲ (RB 1-Jul-2022)
USP Dextran V0 Marker RS

[1] The Gauss-Newton method, modified by Hartley [see D. Hartley *Technometrics* , **3** (1961)], and the G. Nilsson and K. Nilsson method [see G. Nilsson and K. Nilsson J. *Chromat* , **101**, 137 (1974)] are suitable methods. A curve-fitting program capable of nonlinear regression may be used.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
DEXTRAN 70	Ying Han Associate Science & Standards Liaison	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

Chromatographic Database Information: Chromatographic Database

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