C. It complies with the test for molecular-mass distribution (see Tests).

**TESTS**

**Solution S.** Dissolve 7.5 g in carbon dioxide-free water R, heat on a water-bath and dilute to 50 ml with the same solvent.

**Absorbance (2.2.25).** Measure the absorbance of solution S at 375 nm. The absorbance is not more than 0.12.

**Acidity or alkalinity.** To 10 ml of solution S add 0.1 ml of phenolphthalein solution R. The solution is colourless. Add 0.2 ml of 0.01 M sodium hydroxide. The solution is pink. Add 0.4 ml of 0.01 M hydrochloric acid. The solution is colourless. Add 0.1 ml of methyl red solution R. The solution is red or orange.

**Nitrogen-containing substances.** Carry out the determination of nitrogen by sulphuric acid digestion (2.5.9), using 0.200 g and heating for 2 h. Collect the distillate in a determination of nitrogen by sulphuric acid digestion (2.5.9) mixture of 0.5 ml of bromocresol green solution R, 0.5 ml of methyl red solution R and 20 ml of water R. Titrate with 0.01 M hydrochloric acid. Not more than 0.15 ml of 0.01 M hydrochloric acid is required to change the colour of the indicator (110 ppm N).

**Sodium chloride.** Not more than 1.5 per cent. Accurately weigh 3.5 g and dissolve in 100 ml of water R. Add 0.3 ml of potassium chromate solution R and titrate with 0.1 M silver nitrate until the yellowish-white colour changes to reddish-brown.

1 ml of 0.1 M silver nitrate is equivalent to 5.844 mg of NaCl.

**Molecular-mass distribution.** The average molecular mass \( (M_w) \) is 850 to 1150. The fraction with less than 3 units of glucose is less than 15 per cent, the fraction with more than 9 units of glucose is less than 20 per cent.

Examine by size-exclusion chromatography (2.2.30).

**Test solution.** Dissolve 6.0-6.5 mg of the substance to be examined in 1.0 ml of the mobile phase.

**Reference solution (a).** Dissolve 6.0-6.5 mg of dextran 1 CRS in 1.0 ml of the mobile phase.

**Reference solution (b).** Dissolve the content of an ampoule of isomaltooligosaccharide CRS in 1 ml of the mobile phase, and mix. This corresponds to approximately 45 µg of isomaltotriose (3 glucose units), approximately 45 µg of isomaltononaose (9 glucose units), and approximately 60 µg of sodium chloride per 100 µl.

The chromatographic procedure may be carried out using:

- 2 columns, 30 cm long and 10 mm in internal diameter, in series, prepacked with a packing material of dextran covalently bound to highly cross-linked porous agarose beads, allowing resolution of oligosaccharides in the molecular mass range of 180 to 3000, kept at a temperature of 20-25 °C,
- as mobile phase at a flow rate of 0.07-0.08 ml/min maintained constant to ± 1 per cent, a 2.92 g/1 solution of sodium chloride R,
- as detector a differential refractometer.

Inject 100 µl of reference solution (b) and record the chromatogram for definition of the positions of isomaltotriose, isomaltononaose and sodium chloride. Inject 100 µl of the test solution and 100 µl of reference solution (a) and record the chromatograms. Determine the peak areas. Disregard any peak due to sodium chloride.

Calculate the average relative molecular mass \( M_w \) and the amount of the fraction with less than 3 and more than 9 glucose units, of dextran 1 CRS and of the substance to be examined. The test is not valid unless the values obtained for dextran 1 CRS are within the values stated on the label.

\[
M_w = \sum w_i \times m_i
\]

**Heavy metals (2.4.8).** Dilute 20 ml of solution S to 30 ml with water R. 12 ml of this solution complies with limit test A (10 ppm). Prepare the reference solution using lead standard solution (1 ppm Pb) R.

**Loss on drying (2.2.32).** Not more than 5.0 per cent, determined on 5.000 g by drying in an oven at 100-105 °C for 5 h.

**Bacterial endotoxins (2.6.14):** less than 25 IU/g.

**Microbial contamination.** Total viable aerobic count (2.6.12) not more than 10² micro-organisms per gram, determined by plate-count. It complies with the test for Escherichia coli (2.6.13).

**Dextrans 40 for injection**

**DEFINITION**

Dextran 40 for injection is a mixture of polysaccharides, principally of the α-1,6-glucan type. The average relative molecular mass is about 40 000.
Dextran 60 for injection

DEFINITION
Dextran 60 for injection is a mixture of polysaccharides, principally of the α-1,6-glucan type. The average relative molecular mass is about 60 000.

PRODUCTION
It is obtained by hydrolysis and fractionation of dextrans produced by fermentation of sucrose using *Leuconostoc mesenteroides* strain NRRL B-512 = CIP 78.59 or substrains thereof (for example *L. mesenteroides* B-512F = NCTC 10817).

It is prepared in conditions designed to minimise the risk of microbial contamination.

CHARACTERS
A white or almost white powder, very soluble in water, very slightly soluble in alcohol.

IDENTIFICATION
A. Dissolve 1.0 g in *water R*, heating on a water-bath, and dilute to 50.0 ml with the same solvent. The specific optical rotation (2.2.7) of the solution is +195 to +201, calculated with reference to the dried substance.

B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with dextran CRS.

C. It complies with the test for molecular-mass distribution (see Tests).

TESTS
Solution S. Dissolve 5.0 g in *distilled water R*, heating on a water-bath, and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (2.2.1) and colourless (2.2.2, Method II).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of phenolphthalein solution R. The solution remains colourless. Add 0.2 ml of 0.01 M sodium hydroxide. The solution is red. Add 0.4 ml of 0.01 M hydrochloric acid. The solution is colourless. Add 0.1 ml of methyl red solution R. The solution is red or orange.

Nitrogen-containing substances. Carry out the determination of nitrogen by sulphuric acid digestion (2.5.9), using 0.200 g and heating for 2 h. Collect the distillate in a mixture of 0.5 ml of bromocresol green solution R, 0.5 ml of methyl red solution R and 20 ml of *water R*. Titrate with 0.01 M hydrochloric acid. Not more than 0.15 ml of 0.01 M hydrochloric acid is required to change the colour of the indicator (110 ppm N).

Residual solvents. Examine by gas chromatography (2.2.28), using propanol R as internal standard.

Test solution. Dissolve 5 g of the substance to be examined in 100 ml of *water R* and distil. Collect the first 45 ml of the distillate, add 1 ml of a 25 g/l solution of propanol R and dilute to 50 ml with *water R*.

Reference solution. Mix 0.5 ml of a 25 g/l solution of *ethanol R*, 0.5 ml of a 25 g/l solution of propanol R and 0.5 ml of a 2.5 g/l solution of *methanol R* and dilute to 25.0 ml with *water R*.

The chromatographic procedure may be carried out using:
- a stainless steel column 1.8 m long and 2 mm in internal diameter packed with *ethylenevinylbenzene-divinylbenzene copolymer R* (125-150 μm),
- nitrogen for chromatography R as the carrier gas at a flow rate of 25 ml/min,
- a flame-ionisation detector,

maintaining the temperature of the column at 190 °C, that of the injection port at 240 °C and that of the detector at 210 °C. Inject the chosen volume of each solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to ethanol or methanol is not greater than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.5 per cent of ethanol and 0.05 per cent of methanol) and the sum of the areas of any peaks, apart from the peaks corresponding to ethanol, methanol and the internal standard, is not greater than the area of the peak corresponding to the internal standard (0.5 per cent calculated as propanol).

Molecular-mass distribution (2.2.39). The average molecular mass ($M_n$) is 35 000 to 45 000. The average molecular mass of the 10 per cent high fraction is not more than 110 000. The average molecular mass of the 10 per cent low fraction is not less than 7000.

Heavy metals (2.4.8). 12 ml of solution S complies with limit test A (10 ppm). Prepare the standard using lead standard solution (1 ppm Pb) R.

Loss on drying (2.2.32). Not more than 7.0 per cent, determined on 0.200 g by heating in an oven at 105 ± 2 °C for 5 h.

Sulphated ash (2.4.14). Not more than 0.3 per cent, determined on 0.50 g.

Bacterial endotoxins (2.6.14): less than 10 IU/g.

Microbial contamination. Total viable aerobic count (2.6.12) not more than 10² micro-organisms per gram, determined by plate-count. It complies with the test for *Escherichia coli* (2.6.13).

01/2005:1000

**DEXTRAN 60 FOR INJECTION**

**Dextranum 60 ad inyectabile**

**DEFINITION**
Dextran 60 for injection is a mixture of polysaccharides, principally of the α-1,6-glucan type.

The average relative molecular mass is about 60 000.

**PRODUCTION**
It is obtained by hydrolysis and fractionation of dextrans produced by fermentation of sucrose using *Leuconostoc mesenteroides* strain NRRL B-512 = CIP 78.59 or substrains thereof (for example *L. mesenteroides* B-512F = NCTC 10817).

It is prepared in conditions designed to minimise the risk of microbial contamination.

**CHARACTERS**
A white or almost white powder, very soluble in water, very slightly soluble in alcohol.

**IDENTIFICATION**
A. Dissolve 1.0 g in *water R*, heating on a water-bath, and dilute to 50.0 ml with the same solvent. The specific optical rotation (2.2.7) of the solution is +195 to +201, calculated with reference to the dried substance.

B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with dextran CRS.

C. It complies with the test for molecular-mass distribution (see Tests).

**TESTS**
Solution S. Dissolve 5.0 g in *distilled water R*, heating on a water-bath, and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (2.2.1) and colourless (2.2.2, Method II).