Dextran 40

Dextran 40 is a mixture of polysaccharides, principally of the α-1, 6-glucan type. Average relative molecular mass is about 40,000. It is obtained by hydrolysis and fractionation of dextran produced by fermentation of sucrose using strain of *Leuconostoc mesenteroides*.

Category. Plasma substitute.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *dextran 40 IPRS* or with the reference spectrum of dextran.

B. Specific optical rotation (see Tests).

C. Molecular mass distribution (see Tests) the peaks in the chromatogram obtained with the test solution corresponds to peaks in the chromatogram obtained with reference solution.

Tests

Solution A. Dissolve 5 g in *water,* heating on a water bath and dilute to 50.0 ml with*water.*

Appearance of solution. Solution A is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A, add 0.1 ml of *phenolphthalein solution*,the solution remains 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*, the solution is red or orange.

Specific optical rotation (2.4.22). +195.0° to +203.0°, determined on 2.0 per cent w/v solution.

Molecular-mass distribution. Determine by size-exclusion chromatography (2.4.16).

Test solution. Dissolve 6 mg of the substance under examination in 1.0 ml of the mobile phase.

Reference solution (a). Dissolve 6 mg of *dextran 40 IPRS* in 1.0 ml of the mobile phase.

Reference solution (b). A solution containing 0.045 per cent w/v of isomaltotriose (3 glucose units), isomaltononaose (9 glucose units) and 0.060 per cent w/v of sodium chloride in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 10 mm, dextran covalently bound to highly cross-linked porous agarose beads, allowing resolution of oligosaccharides in the molecular mass range of 180 to 3000 (two columns coupled in series),
- temperature: 20-25°,
- mobile phase: a 0.292 per cent w/v solution of *sodium chloride,*
- flow rate: 0.07-0.08 ml per minute,
- differential refractometer,
- injection volume: 100 µl.

Identification of peaks, use the chromatogram obtained with reference solution (b) to identify the peaks due to isomaltotriose, isomaltononaose and sodium chloride.

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 40 IPRS* and of the substance under examination, using the following expression:

$$M_w=\sum w_i \times m_i$$

M_w=average molecular mass of the dextran;
m_i=molecular mass of oligosaccharide *i*;
w_i=weight proportion of oligosaccharide *i.*

Use the following m_i values for the calculation:

Oligosaccharide *i*	m_i
Glucose	180
Isomaltose	342
isomaltotriose	504
isomaltotetraose	666
isomaltopentaose	828
isomaltohexaose	990
isomaltoheptaose	1152
isomaltooctaose	1314
isomaltononaose	1476
isomaltodecaose	1638
isomaltoundecaose	1800
isomaltododecaose	1962
isomaltotridecaose	2124
isomaltotetradecaose	2286
isomaltopentadecaose	2448
isomaltohexadecaose	2610
isomaltoheptadecaose	2772
isomaltooctadecaose	2934
isomaltononadecaose	3096

Inject reference solution (a). The test is not valid unless the values obtained for *dextran 40 IPRS* are within the values stated on the label.

The average molecular mass is35,000 to45,000. The average molecular mass of the 10 per cent high fraction is not more than 1,10,000. The average molecular mass of the 10 per cent low fraction is not less than 7,000.

Nitrogen-containing substances. Not more than 110 ppm of N.

Determine the content of nitrogen, Method A (2.3.30),using 0.2 g and heating for 2 hours. Collect the distillate in a mixture of 0.5 ml of *bromocresol green solution*, 0.5 ml of *methyl red solution* and 20 ml of *water*. 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.

Residual solvents (5.4). Not more than 0.5 per cent *ethanol*, 0.05 per cent *methanol* and sum of solvents other than *ethanol*, *methanol* and *propanol* is not more than 0.5 per cent calculated as *propanol*.

Sulphated ash (2.3.18). Not more than 0.3 per cent,determined on 0.5 g.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1.0 g by drying in an oven at 105° for 5 hours.

Bacterial endotoxins (2.2.3). Not more than 10 Endotoxin Units per g of dextran 40.

Microbial contamination (2.2.9).Total aerobic viable count is not more than 100 CFU per g.

Solubility. Very soluble in *water* and very slightly soluble in *ethanol (95 per cent)*.