Characterization of Dextran

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Introduction

Dextran is a complex polysaccharide that is used in various fields such as pharmaceutical, photographic, agricultural, and food industries. Many glucose molecules ($C_6H_{12}O_6$) are joined into chains of varying lengths with short (mostly one or two sugar units, less than 5%, see fig.1) side branches. Dextran may be synthesized from sucrose by bacteria (e.g. Leuconostoc mesenteroides streptococcus and Streptococcus mutans) and can also be produced by other bacteria and yeasts.

In this application note dextran is characterized using two light scattering techniques at one angle and one concentration. The size and molecular weight of polysaccharides are often used as standards for chromatography calibration.

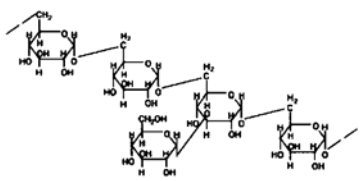

Figure 1: Molecular structure of the repeating dextran unit

Experimental

This polysaccharide polymer exists in many chain lengths and various polymerization fractions of narrow molecular weight are commercially available. Dextran fractions are readily soluble in water and electrolyte solutions to form clear, stable solutions over a wide pH range and up to high concentration. Other solvents exist, notably methyl sulfide, formamide, ethylene glycol, and glycerol, but this polysaccharide is insoluble in monohydric alcohols (e.g. methanol, ethanol, isopropanol) and most ketones (e.g. acetone, 2-propanone).

Three different molecular weight standards were prepared (Dextran-5, 12 and 25 from Pharmacosmos A/S) in deionized water at a concentration of 5 g/L. Samples were subsequently filtered through 0.1 µm pore size membranes (Whatman Anotop) without difficulty. The nominal molar mass of the fractions were provided as 5220, 11600, and 23800 g/mol.

Samples were measured in the Zetasizer Nano S by dynamic light scattering (DLS).

Results

All sample solutions appeared clear to the eye without visible aggregation or tint confirming the dextran is well-dissolved. Even at the smallest size (Dextran-5) the light scattering setup is sensitive enough to provide significant scattering signal for analysis.

Hydrodynamic size

In dynamic light scattering (DLS) intensity fluctuations due to the Brownian motion of the diffusing molecules are analyzed to determine the diffusion coefficient and from this the hydrodynamic size. This technique only requires knowledge of the viscosity of the solvent (water). The experimental result consists of an overall average size, the z-average, and an overall polydispersity index, PDI, obtained from a cumulant or single exponential fitting (data are summarized in table 1).

Sample Name	Z-Average (d.nm)	PDI
5g/L Dextran-5 Mw=5220	3.85 6.7kD	0.17
5g/L Dextran-12 Mw=11600	5.74 13.7kD	0.16
5g/L Dextran-25 Mw=23800	7.97 24.9kD	0.14

Table 1: Hydrodynamic size and estimated molar mass from dextrans

Distribution information may be obtained from a regularization without any assumption of the distribution shape. The size distributions by intensity are overlaid in figure 2.

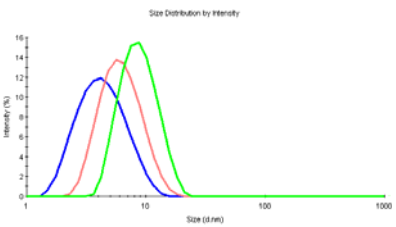

Figure 2: Size distributions by DLS for Dextran-5 (–), Dextran-12 (–), and Dextran-25 (–)

The size distributions and z-average results may be used to estimate the molar mass from an empirical model for linear polysaccharides. The predicted values are listed in table 1.

Polydispersity information

The distribution analysis for polymers is typically provided in terms of Mw/Mn. In light scattering the intensity is the measured quantity, and only indirectly through transformation of the result can a volume or even number distribution be obtained. Caution is generally advised since a

rescaling with d^3 and d^6 can significantly skew the resultant distribution towards small sizes: this should only be undertaken for high quality correlation function data. Since these dextrans are essentially GPC standards the transformations are justified.

For each distribution (intensity, volume, and number) a mean and a polydispersity index may be obtained from the software and these values are listed in table 2. The results show that the polydispersity index for the Dextran-25 is lowest which indicates that Dextrane-25 is the most monodisperse of the standards. This is confirmed from the specifications.

In order to compare the specifications given for these dextrans with the light scattering results the parameter Mw/Mn may be used. This is often called polydispersity in polymer chemistry. Here, the values for Mw/Mn of 1.64, 1.54, 1.47 (DLS) are in reasonable agreement with 1.60, 1.43, 1.30 (GPC) for Dextran-5, Dextran-12, and Dextran-25. The expected empirical dependence is:

$$PDI \sim (Mw/Mn - 1)^2$$

Sample	Intensity		Volume		Number	
	size (d.nm) MW est	PDI	size (d.nm) MW est	PDI	size (d.nm) MW est	PDI
Dextran-5 Mw=5220	4.63 9.3kD	0.21	2.76 3.6kD	0.17	2.07 2.2kD	0.087
Dextran-12 Mw=11600	6.54 17.4kD	0.16	4.41 8.5kD	0.14	3.48 5.5kD	0.082
Dextran-25 Mw=23800	9.09 31.6kD	0.12	6.61 17.8kD	0.12	5.36 12.1kD	0.072

Table 2: Size and estimated molar mass from Dextrans, distribution analysis

Measured molecular weight

In static light scattering (SLS) the absolute MW is typically determined by detecting the scattered intensity from several known sample concentrations. Here, a single concentration method and prior knowledge of the second Virial coefficient of dextran in water (from Malvern Application Note MRK577-01: 0.011 mol mL / g^2) is used: The measured intensity (from table 1) is adjusted for water (25Kcps/s), scaled with toluene (237.1 Kcps/s), and Kc/R computed using dn/dc=0.150 mL/g [#].

The molecular weights obtained from SLS (listed in table 3) are acceptably close to the nominal Mw from the manufacturer.

Sample	Conc'n	Residual intensity (kCts/s)	Kc/R (1/Da)	Revised Kc/R, c→0 (1/Da)	SLS MW (Da)
Dextran-5 Mw=5220	5 g/L	71.8	2.50E-04	1.95E-04	5130
Dextran-12 Mw=11600	5 g/L	140.7	1.27E-04	7.25E-05	13800
Dextran-25 Mw=23800	5 g/L	178.9	1.00E-04	4.52E-05	22100

Table 3: Static light scattering results for Dextrans (single concentration)

Summary

Dextran was characterized by dynamic and static light scattering to obtain molecular weight and polydispersity information. Results are in good agreement with sample specifications obtained from gel permeation chromatography.

Zetasizer Nano system

The Zetasizer Nano system from Malvern Instruments is the first commercial instrument to include the hardware and software for combined dynamic, static, and electrophoretic light scattering measurements, providing the researcher with a wide range of sample properties, including the size, molecular weight, and zeta potential. The system was specifically designed to meet the low concentration and sample volume requirements typically associated with pharmaceutical and biomolecular applications.

[#] Refractive index increment data-book for polymer and biomolecular scientists, A. Theisen, C. Johann, M.P. Deacon, S.E. Harding, Nottingham Press (2000)

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