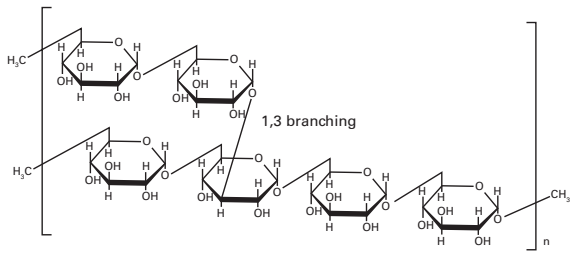
TOSOH

Application Note

Monitoring Structural Changes in Polysaccharides using SEC-MALS

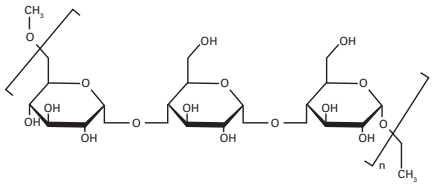
Dextran is one of the most common representatives of polysaccharides, a macromolecule consisting of α-1,6 linkages between glucose units. In addition, α-1,3 (and infrequently α-1,2) linkages can be present, thus creating branches on the main glycan chain (see *Figure 1*). These materials have a remarkable diversity in physicochemical properties due to the variation in chain length and degree of branching. Dextran's commercial applications are typically found in the food and pharmaceutical industry, such as vaccines, eye medicines, organ preservation, and blood cell separation. They are also used as blood plasma surrogates.

Figure 1. Structure of dextran.



Pullulan is another widely studied type of polysaccharide. It consists of maltotriose units, made of three glucose monomers with α-1,4 linkages, which are connected by α-1,6 glycosidic bonds (see *Figure 2*). The coexistence of both types of glycosidic bonds generate an intermediate structure between dextran and amylose. This unique linear structure provides the specific structural flexibility and solubility of pullulan, resulting in distinct film- and fiber-forming characteristics which are not exhibited by other polysaccharides. Pullulans have numerous uses in the food, manufacturing, electronic, and pharmaceutical industries, such as wound-healing compositions, pharmaceutical coatings, oral care products, and non-toxic conjugates for vaccines.

Figure 2. Structure of pullulan.



As the most abundant natural biopolymer, polysaccharides' unique chemical and physical properties, as well as their excellent biocompatibility make them materials of choice in many industries. Due to their wide application range and the complexity of their structure, such polymers need to be examined very thoroughly to fully understand their molecular characteristics. For example, to study their diffusion properties, the size of the molecules is an important parameter. Also, the size of dextran is by far the most important determinant of red blood cell aggregation, where low size molecules inhibit aggregation while larger molecules promote aggregation. In addition, a great variety of conformations and branching behaviors make specific polysaccharides either suitable or problematic for certain applications.

This application note explains how size exclusion chromatography (SEC) coupled with multi-angle light scattering (MALS) can be used for the determination of structural changes in polysaccharides, with pullulans and dextrans as examples.

Experimental Conditions

System:	EcoSEC Elite® (HLC-8420) GPC system
Columns:	2 × TSKgel® GMPW$_{XL}$
Mobile phase:	Water + 0.01 mol/L $NaNO_3$ and 0.02 % NaN_3
Flow rate:	0.7 mL/min
Detectors:	Refractive index (RI) and LenS$_3$® MALS detector

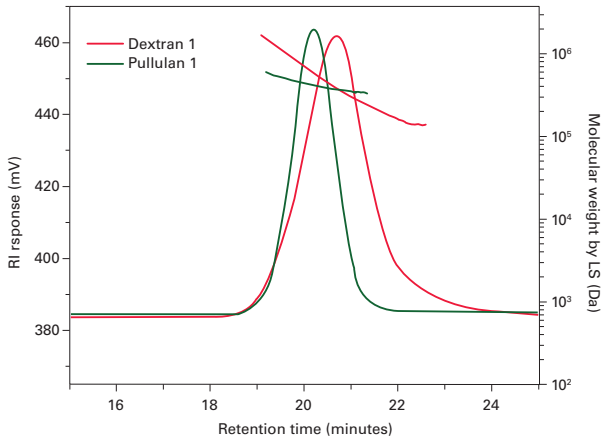
The system was calibrated using polyethylene oxide (SE-5) with a molecular weight (MW) of 44 kDa. The concentration of the SE-5 solution in the eluent was 1.6 mg/mL and a volume of 100 µL was injected onto the columns. The specific refractive index (dn/dc) of the SE-5 is 0.132 mL/g.

Results and Discussion

Figure 3 shows the RI chromatogram obtained for pullulan 1 and dextran 1. From the elution profiles, pullulan 1 elutes earlier than dextran 1 which indicates that the hydrodynamic volume (V_h) of dextran 1 is smaller than pullulan 1. However, the MW distribution (based on light scattering) of the samples showed that dextran 1 has a higher MW compared to the earlier eluted pullulan 1.

From the elution and MW profiles of these samples, we can conclude that the mass-to-size ratio (density) of dextran 1 is higher compared to that of pullulan 1, suggesting that dextran 1 has an increased molecular density due to branching.

Figure 3. Chromatogram of dextran 1 and pullulan 1 samples.



To further investigate the assumption, a set of linear pullulan standards with MW ranging from 21 kDa to 915 kDa was analyzed in the same experimental conditions. Using the SECview software, the radius of gyration (R_g) and the MW of the pullulan standards were determined and reported in *Table 1*. It is noticeable that R_g values were obtained for all the standards, even the smaller ones. Technically, traditional MALS detectors cannot detect the angular dependence of scattered light to measure R_g for sizes below 12 nm, at best. However, the novel design of the LenS3 MALS detector extends its R_g measurement range to much smaller polymers. Here, an R_g of 5.1 nm for the lowest MW pullulan standard (21 kDa) was obtained.

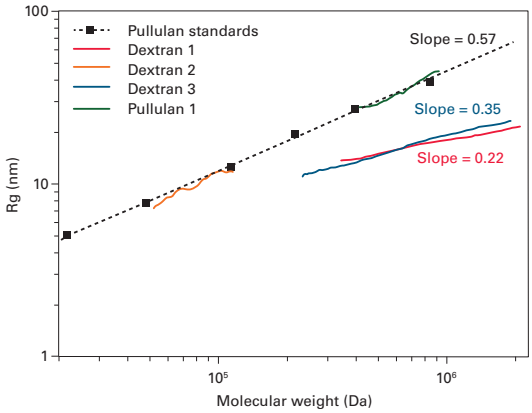
Table 1. MW and R_g of the dextran and pullulan samples.

Samples	M_w	CV (%)	R_g	CV (%)
Pullulan standards	21,749	0.48	5.1	4.3
	47,778	0.36	7.8	0.62
	113,042	0.20	12.6	1.2
	217,515	0.21	19.4	1.2
	399,466	0.20	27.3	0.6
	839,454	0.20	38.9	0.67
Pullulan 1	399,466	0.20	27.3	0.6
Dextran 1	487,449	1.15	17.3	1.33
Dextran 2	56,873	0.64	6.3	2.08
Dextran 3	284,999	0.67	13.8	0.51

M_w = weight average molecular weight, CV=coefficient of variation (triplicate injections)

The conformation plot in *Figure 4* shows that the correlation between the R_g and MW of the pullulan standards is linear with a slope of 0.57 which is typically expected for random coil polymers in good solvents. The broader distribution of pullulan 1 falls exactly on that extrapolated conformation plot.

Figure 4. Conformation plot of pullulan and dextran.



In addition to the linear pullulan standards, dextrans with different MW distributions were also analyzed and the results are presented in *Table 1* and *Figure 4*. The R_g of the low MW dextran (dextran 2) across its entire distribution overlays perfectly with the pullulans' conformation plot. This relationship is an indicator of molecular density, suggesting that the low MW dextran has the same linear structure as the pullulans. Conversely, the R_g to MW relationships of dextran 1 and dextran 3 have a slope of 0.22 and 0.35, respectively. These are much smaller slope values than the linear pullulans, suggesting a comparatively denser structure.

The linear pullulans form a random coil structure in the eluent. In the case of dextrans, there is possible long chain branching as explained in the introduction, especially in the high MW dextrans. As a result, a more compact random coil structure is formed while dissolving in the eluent. Due to this compact structure, the size of the molecule is smaller and thereby elutes at a higher elution time from the column.

Conclusion

This study demonstrates that structural differences in polymers can be investigated in depth by SEC-MALS analyses. In the given example, higher MW dextrans tend to exhibit increased branching on their backbone, leading to the formation of a more compact structure in solution. Ultimately, this results in higher retention volumes and lower R_g values compared to linear pullulans of similar MW. In practice, elucidating structural changes in the low MW and low R_g region requires a light scattering instrument with high sensitivity and capable of detecting very slightly anisotropic scattering, such as the LenS3 MALS detector.

Featured Products

Part #	Description
ECOSYS Elite-RI	EcoSEC Elite GPC System
40000	LenS$_3$ Multi-Angle Light Scattering Detector
08025	TSKgel GMPW$_{XL}$, 13 µm, 7.8 mm ID x 30 cm
06212	SE-5, 3.9 x 10^4 Da