High resolution Saccharide and Polysaccharide Analysis using Small Particles

Application Note Food Analysis/Pharmaceutical Analysis

Author
Dr. Günter Reinhold
contact: DHeld@pss-polymer.com

The PSS SUPREMA column with a reduced particle size of 5 µm offers a significant improvement in performance compared to traditional 10 µm materials and provides outstanding additional resolution, especially in the low molecular weight area, which is a major consideration when analyzing oligomeric polysaccharides.

Introduction
Polysaccharides are very important in nature, occurring in food (starches in rice, wheat etc.) and plants (cellulose). Some polysaccharides are also produced commercially e.g. Dextrans, which are manufactured through the fermentation of sugar solutions. These are higher molar mass polysaccharides.

Dextrans are used in clinical and technical applications, where molecular weight is critical in determining the properties of the final product. Accurate determination of the molecular weight distribution is vital.

On the other hand, low molar mass saccharides are also very common e.g. in food, such as fruits, honey and sweets. Examples for low molar mass sugars are mono- (glucose, fructose), di- (lactose, isomaltose, trehalose) and trisaccharides (maltotriose, isomaltotriose). The separation and identification of low molar mass polysaccharides is a challenge as the compounds have the same chemical formula and only small differences in structure, e.g disaccharides maltose, isomaltose, gentiobiose cellobiose and trehalose $C_{12}H_{22}O_{11}$.

System Requirements

	Conditions
Pump	PSS SECcurity GPC1260 isocratic pump • flow rate [mL/min]: 0.25 • mobile phase: aqueous, 0.1 M $NaNO_3$
Injection system	PSS SECcurity GPC1260 Autosampler • injection volume variable
Columns	• PSS SUPREMA precolumn (8*50mm) • PSS SUPREMA 5µ 100 Å, 100 Å, 100 Å (8*300mm each)
Loading	• 4.0 mg/mL, 5 µL injection volume
Detectors	• Refractive index PSS SECcurity 1260 RI
Software	PSS WinGPC UniChrom • optional for 21CFR11 compliance: Compliance Pack • optional: modules for mass spectrometry, 2D, viscometry, light scattering, end group analysis

PSS Polymer Standards Service GmbH
In der Dalheimer Wiese 5
55120 Mainz | Germany
Phone +49 6131 96239-0
Fax +49 6131 96239-11
E-Mail info@pss-polymer.com
Web www.pss-polymer.com

Polymer Standards Service-USA, Inc.
160 Old Farm Rd, Suite A
Amherst | MA 01002 | USA
Phone +1 413 835-0265
Fax +1 413 835-0354
E-Mail usa@pss-polymer.com
Web www.pss-polymer.com

Procedure, Results & Discussion

A high resolution and therefore a good separation is necessary for a precise analysis. This is particularly important when new analytical LC coupling methods like GPC/SEC-ESI-MS are used, as the MS detector requires the columns to have a much higher resolution power within an overall smaller column volume.

The reduction of the particle size results in a higher resolution (compare Figure 1). Therefore PSS developed the PSS SUPREMA 5 µm columns, to replace the standard columns with larger particle sizes traditionally used in aqueous GPC/SEC.
PSS SUPREMA columns can be used for numerous neutral and anionic aqueous applications in the molecular weight area between 100 Da to around 5 million Da. The columns are available in analytical (ID: 8mm) and micro (ID: 4.6mm) dimensions with different porosities. Linear or mixed columns are also available.

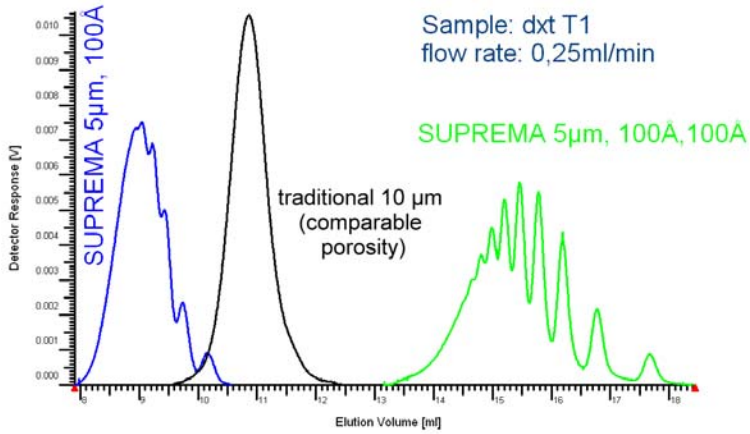

Fig. 1: Comparison of the separation of a low molar mass Dextran T1 on a traditional aqueous column (black curve) compared to separations on one (blue) and two (green) SUPREMA 5 µm.

The analysis of dextran T1 (Figure 2) shows the separation power when a combination of three SUPREMA 5µm 100 Å columns is used. The oligomers in the low molecular weight are able to be resolved up to P10. As a reference a glucose separation is overlaid.

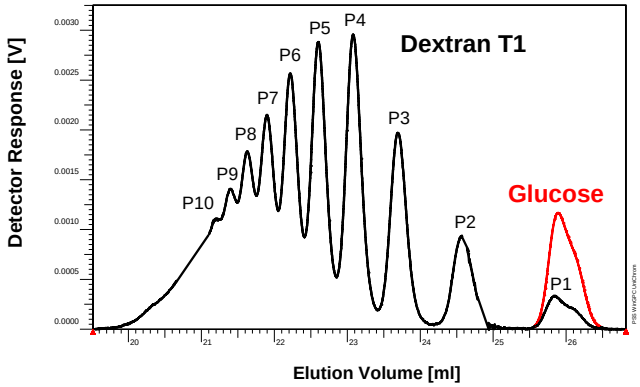

Fig. 2: Overlay of elugrams of a glucose (red curve) with a low molar mass Dextran T1 (black curve)

PSS Polymer Standards Service GmbH
In der Dalheimer Wiese 5
55120 Mainz | Germany
Phone +49 6131 96239-0
Fax +49 6131 96239-11
E-Mail info@pss-polymer.com
Web www.pss-polymer.com

Polymer Standards Service-USA, Inc.
160 Old Farm Rd, Suite A
Amherst | MA 01002 | USA
Phone +1 413 835-0265
Fax +1 413 835-0354
E-Mail usa@pss-polymer.com
Web www.pss-polymer.com

The analysis of different disaccharides (Figure 3) shows the ability to separate compounds with the same chemical formula and with only small differences in structure and hence size in solution.

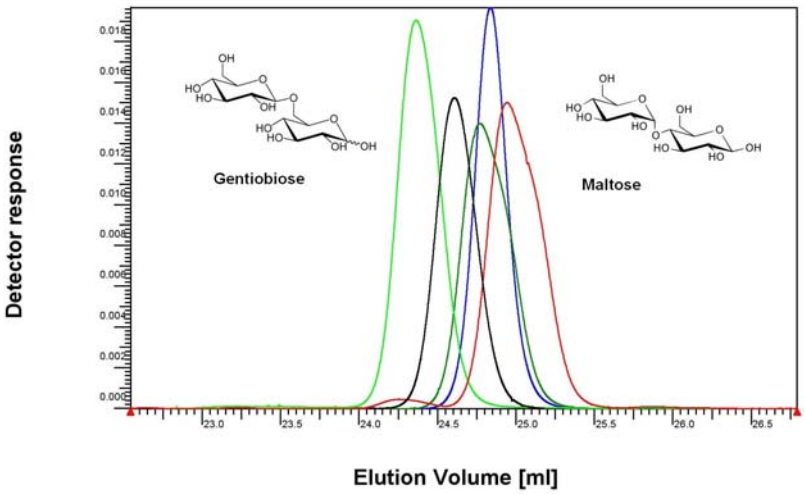

Fig. 3: Overlay of elugrams of isomaltose (black), maltose (red), gentiobiose (green), cellobiose (dark green) and trehalose (blue).

PSS Polymer Standards Service GmbH
In der Dalheimer Wiese 5
55120 Mainz | Germany
Phone +49 6131 96239-0
Fax +49 6131 96239-11
E-Mail info@pss-polymer.com
Web www.pss-polymer.com

Polymer Standards Service-USA, Inc.
160 Old Farm Rd, Suite A
Amherst | MA 01002 | USA
Phone +1 413 835-0265
Fax +1 413 835-0354
E-Mail usa@pss-polymer.com
Web www.pss-polymer.com