# The Characterization of Sugar Beet Pectin using the EcoSEC® GPC System Coupled to Multi-Angle Light Scattering, Quasi-Elastic Light Scattering, and Differential Viscometry

EcoSEC GPC System
APPLICATION NOTE

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## Abstract

The need to increase the use of low valued co-products derived from the processing of sugar beets has prompted the investigation of the structure of the pectin extracted from sugar beet pulp. The characterization of sugar beet pectin is essential as it has the potential to be used in the production of industrial products, *e.g.*, as an emulsifying agent in food systems. This added use of sugar beet pectin should be of help to sugar beet growers and processors by increasing the demand and value of their by-product without increasing the cost of sugar to the consumer. Here we discuss the characterization of sugar beet pectin utilizing the EcoSEC GPC System with an internal dual-flow differential refractive index detector and UV detector coupled to multi-angle light scattering, quasi-elastic light scattering, and differential viscometry. Implementing this multi-detector SEC technique allowed for the determination of the molar mass averages, in a calibrant-independent fashion, as well as several sizing parameters.

## Introduction

An estimated 2 million tons of dry sugar beet pulp is generated annually by U.S. industries as a result of the extraction of sugar from sugar beets[1]. Currently sugar beet pulp is mainly dried and sold as low-value animal feed at little profit because of the costly energy required to dry it for storage and shipment. Sugar beet pulp, especially the high molar mass pectin portion, has the potential to be an enormous untapped source of a valuable polysaccharide for U.S. industry. The need to increase the utilization of low-value co-products derived from the processing of sugar beets has prompted the investigation of the structure of the pectin extracted from sugar beet pulp.

Sugar beet pulp on a dry weight basis is composed of about 67% plant cell wall polysaccharides, 19% of which are pectin, 21% pectin-associated arabinan, and 24% cellulose[1], all of which can potentially add value to the pulp if isolated and characterized. The pectin in sugar beets has different chemical features than that from other sources of pectin, i.e. citrus, as the former tends to have a higher degree of acetylation, a higher natural sugar content, and contains feruloyl groups. Furthermore, unlike citrus pectin which is currently used as a gelling and thickening agent, sugar beet pectin (SBP) has very poor gelling properties, thus the isolation and characterization of it could result in new applications, especially in the production of industrial products. Thus far, SBP has demonstrated the potential to be used as an emulsifying agent in food systems, as it has been found to reduce the interfacial tension between oil and water phases[1]. The capability of SBP to reduce surface tension has been attributed to the presence of acetyl groups and hydrophobic proteins in SBP preparations[2].

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In order to better understand the ability of SBP to act as an emulsifier, we have investigated the structure of SBP using size exclusion chromatography coupled to a multiplicity of physical detection methods, namely multi-angle light scattering (MALS), quasi-elastic light scattering (QELS), multi-wavelength UV, differential refractometry (RI), and differential viscometry (VISC), and corroborated these results with results from atomic force microscopy[1].

## Experimental Conditions

Sugar beet pectin was prepared using microwave-assisted flash-extraction as described in reference 1. Size exclusion chromatography analysis of the sugar beet pectin was performed on a system consisting of an EcoSEC GPC System (Tosoh Bioscience, King of Prussia, PA) with an internal dual-flow differential refractive index detector and UV detector connected in series to a HELEOS II MALS photometer (Wyatt Technology Corp., Santa Barbara, CA), a QELS photometer (Wyatt), and a ViscoStar differential viscometer (Wyatt). The UV absorbance was monitored at wavelenghts of 310, 278, and 250 nm. The solvent and mobile phases were water with 0.05 M $NaNO_3$ and 0.01% $NaN_3$, at a flow rate of 0.7 mL/min. 200 µL injections of 1 mg/mL solutions were injected onto a column bank consisting of three TSKgel GMPW$_{XL}$ columns (30 cm x 7.8 mm) with a particle size of 13 µm obtained from Tosoh Bioscience. These mixed-bed columns have a separation range, based on polyethylene oxides, of 1000 to 8 x $10^6$ g/mol. Detectors, pump oven, and column oven were maintained at 35°C. Data acquisition and processing were performed using Wyatt's ASTRA 5.3.4.16 software.

The HELEOS II detector was normalized in-house using a pullulan standard, with a molar mass of 47,300 g/mol, while calculation of interdetector delays and interdetector band broadening correction were performed using BSA. Calibration of the MALS unit was performed using toluene. The $\partial n/\partial c$ of sugar beet pectin was determined previously to be 0.130 mL/g.

## Results and Discussion

As described above, the EcoSEC GPC System equipped with TSKgel SEC columns was coupled to a train of MALS, QELS, UV, RI, and VISC detectors to determine the molar mass and size of sugar beet pectin. The results of the experiments are given in *Table 1*. *Figure 1* shows the chromatograms of the sugar beet pectin, as monitored by the individual detectors. The SEC elution profile of the SBP, as measured by both of the concentration-sensitive detectors, RI and UV, displays a distinct bimodal distribution.

**Table 1.** Physical Properties of Sugar Beet Pectin

| | Detection Method | | | |
|---|---|---|---|---|
| | RI | UV @ 250 nm | UV @ 278 nm | UV @ 310 nm |
| $M_W$ (g/mol)[a] | 1.098 x $10^6$ | 1.097 x $10^6$ | 1.147 x $10^6$ | 1.187 x $10^6$ |
| | (0.003 x $10^6$)[b] | (0.004 x $10^6$) | (0.004 x $10^6$) | (0.002 x $10^6$) |
| $R_{G,z}$ (nm)[a] | 43 (1) | 43 (1) | 45 (1) | 42 (1) |
| $R_{H,z}$ (nm)[c] | 53 (1) | 43 (1) | 45 (1) | 44 (1) |
| $[\eta]_W$ (dL/g) | 3.5 (0.1) | 3.4 (0.1) | 3.5 (0.1) | 3.5 (0.1) |

[a] with MALS; [b] Standard Deviation; [c] with QELS

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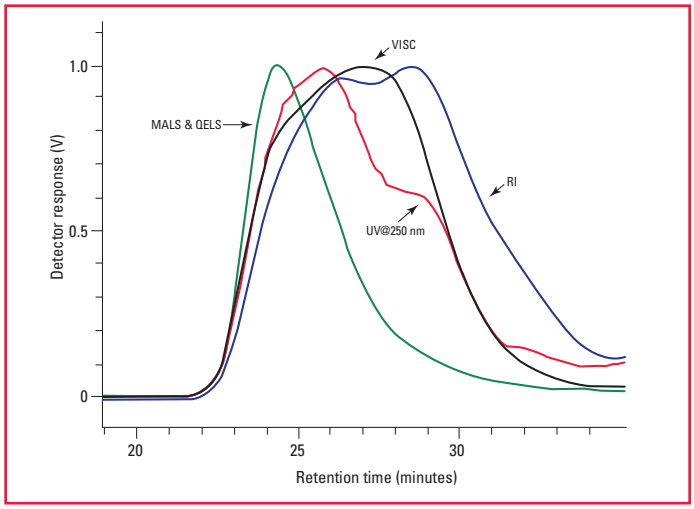
**Figure 1.** SEC elution profile of sugar beet pectin as monitored by MALS & QELS (green), RI (blue), UV @ 250 nm (red), and VISC (black) at 0.7 mL/min.

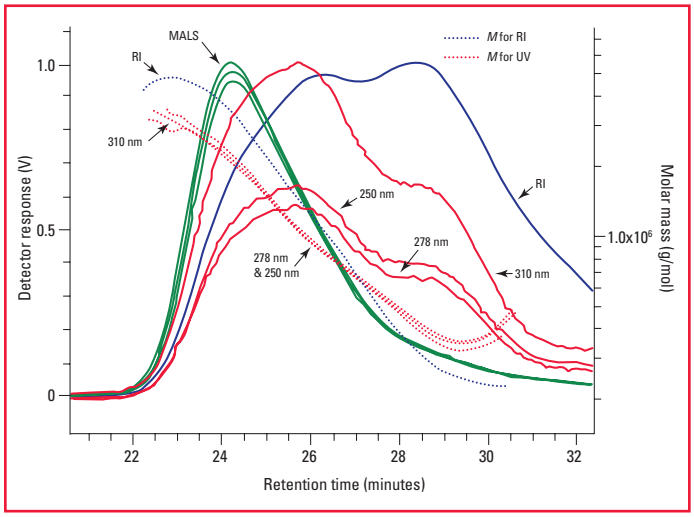



The weight-average molar mass values $M_W$, given in *Table 1*, are calculated via:

$$M_w = \frac{\sum_i c_i M_i}{\sum_i c_i}$$

where, at each elution slice *i*, $c_i$ is the concentration of the analyte provided by the concentration-sensitive detector and $M_i$ is the molar mass of the analyte provided by the MALS detector after correction for interdetector delay. As indicated in *Table 1*, the weight-average molar mass for the SBP was determined to be approximately 1.1 x $10^6$ g/mol and shown to vary less than 7% amongst the four concentration-sensitive detectors (UV @ 310, 278, and 250 nm and RI). Evidence of molar mass polydispersity of SBP is seen in *Figure 2*, where *M* is plotted across the SEC elution for each concentration sensitive detector. From the detector response for the four concentration-sensitive detectors it appears as if the chromatogram is bimodal. Additionally, if one observes the molar mass of the SBP as a function of SEC elution volume, it can be seen that the molar mass of the SBP decreases by an order-of-magnitude with increasing elution volume, an indication the SBP is polydisperse with respect to molar mass. It should also been noted that if one compares the molar mass distributions of the four concentration-sensitive detectors, the molar mass of the SBP is higher at lower elution volumes and lower at larger elution volumes via the RI detector than via the UV detector. The difference in molar masses between the various detection methods is an indication that the particles with higher molar masses have fewer UV absorbing molecules associated with them than their lower molar mass counterparts.

**Figure 2.** SEC elution profile of sugar beet pectin using four concentration-sensitive detectors (UV @ 310, 278, and 250 nm and RI) and molar mass distributions across the elution profiles as determined by MALS.




The addition of the two light scattering detections, MALS and QELS, and viscometry detection to the EcoSEC GPC System not only allows for the determination of the molar mass of SBP in a calibrant-independent fashion but also for the determination of polymeric size and intrinsic viscosity. The radius of gyration $R_G$ and the hydrodynamic radius $R_H$ for the SBP were determined via MALS and QELS, respectively, and are given in *Table 1*. The difference in the value of $R_G$ for SBP as determined by the four concentration-sensitive detectors is minimal, ± 3 nm. Conversely, the difference in the value of $R_H$ for SBP as determined by the RI detector compared to the three UV wavelengths varies by 10 nm, a direct reflection of the dependence of concentration, as determined by the concentration-sensitive detector, on the calculation of $R_H$.

## Conclusions

The EcoSEC GPC System with refractive index and UV detection was coupled to MALS, QELS, and VISC detection to characterize the physicochemical properties of sugar beet pectin. The sugar beet pectin was determined to be polydisperse with respect to molar mass and to have a weight-average molar mass value around 1.1-million g/mol. Two polymeric size parameters were also determined, $R_G$ and $R_H$, as well as the intrinsic viscosity. The coupling of the EcoSEC GPC System to external detectors was successful for the analysis of complex macromolecules based on molar mass, size, and intrinsic viscosity.

## References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2. J. Leroux, V. Langendorff, G. Schick, V. Vaishnav, J. Mazoyer Food Hydrocolloids 17, 455-462 (2003).



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