WYATT TECHNOLOGY

APPLICATION NOTE

AN1310: SEC-MALS analysis of fluorescent lignosulfonate polymers

Introduction

Lignins are ubiquitous cell wall components found in all vascular plants. They are biopolymers whose abundance in nature is second only to cellulose. Derivatives of lignins, such as lignosulfonates, have many different industrial applications that run the gamut from kraft paper manufacturing to special automotive filters.

Lignin branching, mass and size distribution play an important role in determining the application for which the lignin will be best suited. We find multi-angle light scattering used in conjunction with HP-SEC (SEC-MALS) to be an excellent tool for characterizing these polymers, measuring molar mass and radius, their distributions and moments. MALS does not depend on column calibration with linear polymers (that do not elute in the same manner as branched polymers) and constitutes an absolute method for determining these properties.

Lignins exhibit significant absorbance of light, a substantial fraction of which is re-emitted at longer wavelengths; i.e. as fluorescent radiation. These phenomena of absorbance and fluorescence present a significant challenge: they adversely impact the detected light scattering signals, making them almost impossible to interpret for most light scattering instruments. Absorbance of scattered light results in decreased signals, while subsequent fluorescence increases the signals, each in an unpredictable manner.

With the DAWN® multi-angle light scattering (MALS) detector, however, the forward laser monitor can be used to measure the effective transmittance of the laser light used to perform light scattering analysis; ASTRA® chromatography software makes use of the information to correct the analysis in order to take into account the loss of laser intensity prior to reaching the detection volume. In addition, narrow band-pass filters can be placed in front of specific photodiodes to block any fluorescent photons and transmit only the scattered photons.

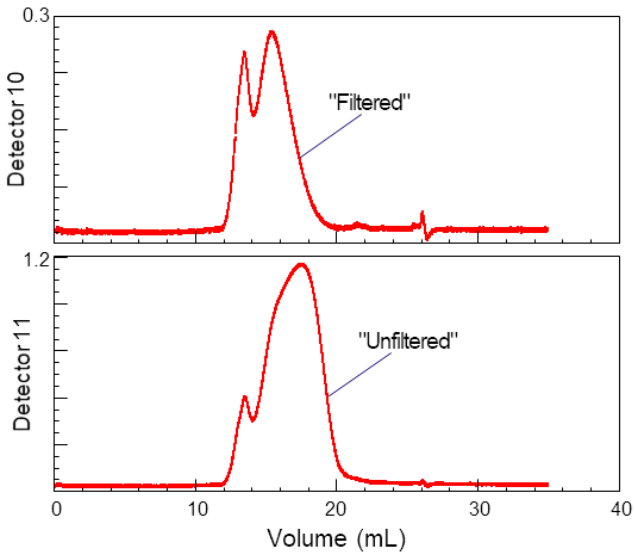


Figure 1. The LS signal is corrected for fluorescence in Detector 10 using an interference filter. Note the marked difference in scattering magnitude versus Detector 11, which has no filter.

Results

The magnitude of the unfiltered fluorescence on the light scattering signals of a lignosulfonate sample is shown in Fig. 1. In this experiment, even-numbered detectors (#2—18) were fit with interference filters which remove light at all wavelengths, except that of the incident laser. The odd-numbered detectors had no filters. The detectors with filters showed a markedly decreased signal when compared to the detectors which had no filters.

An Optilab® differential refractometer measures the polymer concentration at each eluting fraction in order to

complete the analysis of molar mass. In Fig. 2 the results from unfiltered detectors and filtered detectors are contrasted. The apparent molar mass values determined without filters do not follow the expected log(*M*) dependence with elution volume, while the use of fluorescence-blocking filters restores the expected behavior and provides correct results. This graph underscores the importance and necessity of correction for fluorescence when using light scattering detectors to determine the absolute molar masses and sizes of fluorescent polymers like lignins.

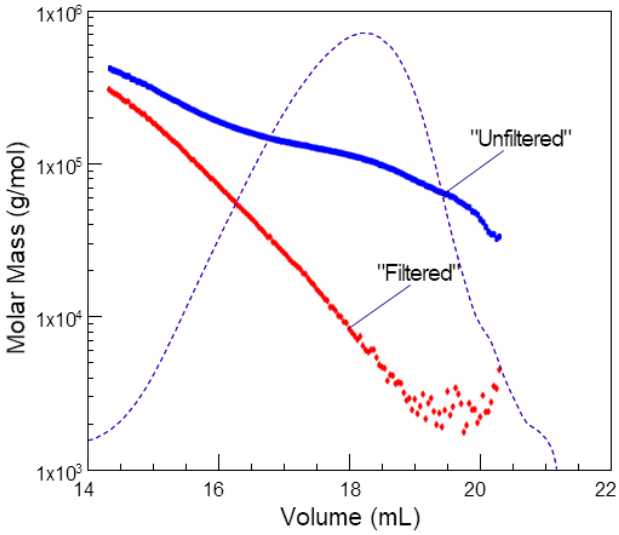
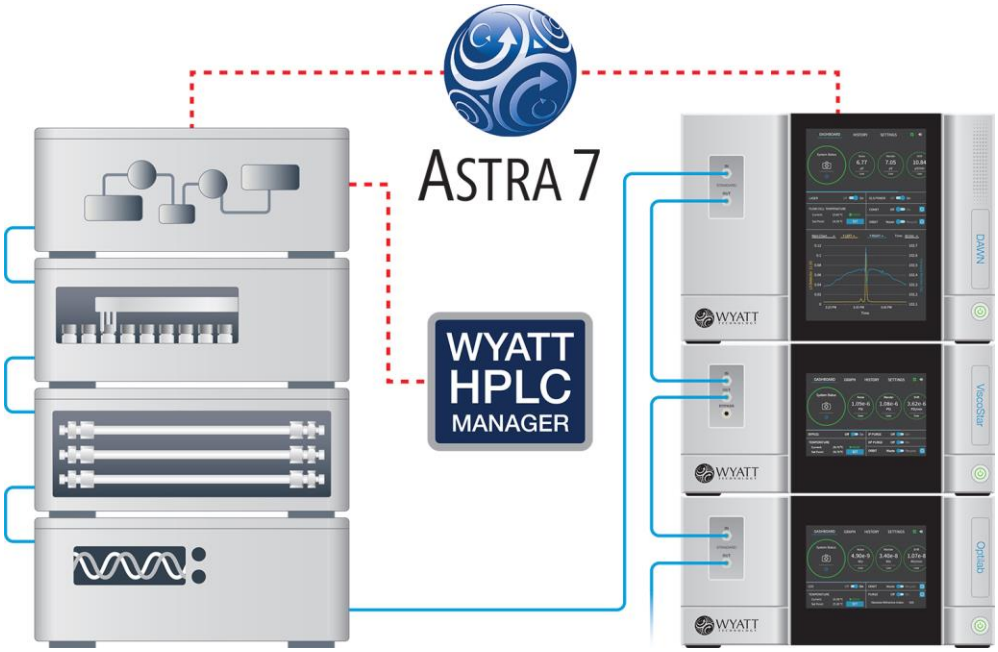


Figure 2. Two very different molar mass distributions are obtained depending on whether odd detectors (no filters) or even detectors (filtered) are computed. The RI trace is overlaid in the background.



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