

# AN1313: Monitoring high-shear molecular weight reduction of polysaccharides by SEC-MALS

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## Summary

Controlling the molecular weight of polysaccharides is crucial in conjugated vaccine production to improve efficacy, reproducibility and product quality. This application note describes the use of a high-shear processor and shear jetting to control the degradation of polysaccharides. It examines how variations in concentration and the number of passes affect the final product. Throughout the process, [multi-angle light scattering coupled to size-exclusion chromatography \(SEC-MALS\)](#) was deployed as a characterization technique. Shear jetting was found to effectively reduce molecular weight and polydispersity, resulting in a more uniform product that meets drug substance quality requirements.

## Introduction

Polysaccharides are highly diverse and heterogeneous [polymers](#) composed of repeating sugar units, with molecular weights (MW) generally ranging from thousands to millions of Daltons in a single sample. For polysaccharide vaccine drug substance, the overall MW must be reduced from its initial state prior to conjugation with adjuvants. In addition to lower MW, low polydispersity of the final substance is beneficial in terms of potency and quality.

One common method for MW reduction is chemical hydrolysis. However, this treatment tends to break the polymer chain randomly, increasing polydispersity and reducing the quality of the final product. It can also adversely affect the chemical structure.

An alternative to chemical hydrolysis is mechanical degradation, such as shear stress, to fracture the polymer. Shear stress tends to break polymer molecules in the middle, resulting in a decrease in polydispersity.

Precise control over polysaccharide degradation and accurate characterization are essential in vaccine production to ensure consistency and potency. In this study, [Fina Biosolutions, LLC](#) utilized the [Dyhydromatics® ShearJet® HL60](#) to reduce the molecular weight of a polysaccharide polymer and confirm the expectation of combined MW and polydispersity reduction. SEC-MALS served to characterize the product following each homogenization cycle, enabling thorough understanding of the effect of concentration and the number of cycles on these key product quality attributes.



## Terminology:

- $M_n$ : number-average molar mass, calculated from the molar mass distribution determined by SEC-MALS
- $M_p$ : molar mass at SEC elution peak, determined by SEC-MALS
- $M_w$ : weight-average molar mass  $M_w$ , which can be calculated from the SEC-MALS molar mass distribution or measured directly by batch MALS.
- $\mathcal{D}$  (dispersity): polydispersity index (PDI) calculated as the ratio of  $M_w/M_n$ .

## Materials and Methods

### Experimental Conditions

A native polysaccharide sample with an initial weight-average molar mass ( $M_w$ ) of 574 kDa was solubilized overnight at a concentrations of 10 mg/mL, 50 mg/mL and 100 mg/mL in water.



Figure 1. The ShearJet HL60 electric hydraulic processor. The touchscreen display shows relevant information in real time.

The mechanical degradation experiment utilized a Dyhydromatics ShearJet HL60 (Figure 1) outfitted with a Type 87.1L Reaction Chamber<sup>®</sup>, which features an L-shaped flow path. The Reaction Chamber module (Figure 2) consists of microchannels the width of a human hair, through which liquid is forced under high pressure.

The ShearJet HL60 runs sample sizes from 3 mL to 100 mL in distinct passes or continuous runs. Its small dead volume and the absence of a priming valve lead to reduced waste compared to typical homogenizers.



Figure 2. At the heart of the ShearJet instrument is the Reaction Chamber, consisting of microchannels the width of a human hair.

The sample is subjected to forces of shear and impact as the polymers navigate the flow path. The fluid spends only a fraction of a second in the Reaction Chamber module and is then efficiently cooled to protect the sample. The process was conducted at a pressure of 30 kpsi with cooling to maintain a temperature of 5°C. Samples underwent five passes through the Reaction

Chamber and aliquots were collected for analysis after each pass. Unlike homogenizers, there are no moving parts within the chamber, so 100% of the formulation experiences the same forces, resulting in tight MW distributions.



Figure 3. The DAWN™ MALS photometer and Optilab™ dRI detector couple to standard HPLC systems to perform SEC-MALS analysis. Wyatt's ASTRA software controls both Waters HPLC modules and the Wyatt detectors for seamless polymer characterization.

### Characterization by SEC-MALS

SEC-MALS combines size-exclusion chromatography with multi-angle light scattering to measure molecular weight distributions, then calculate a polymer's weight-average molecular weight ( $M_w$ ) and polydispersity ( $\mathcal{D}$ ). Unlike traditional analytical SEC, which estimates MW based on column calibration curves determined with molecular standards, SEC-MALS directly provides these values at each eluting slice from [first-principles physical equations](#).

SEC was performed using an industry-standard HPLC system (Figure 3). Data from [DAWN MALS](#) and [Optilab dRI](#) instruments positioned downstream of the SEC column were collected and analyzed using [ASTRA™](#) software.

The DAWN includes 18 angles of scattering detection for the broadest and most accurate measurements of MW and root-mean-square radius ( $R_g$ ), while the Optilab measures both differential and absolute refractive index with industry-leading sensitivity and range. The matching wavelengths of these instruments are essential for accurate MW determination.

## Results

### Effect of the number of passes through the High-Shear processor

Native polysaccharide solutions were prepared at a concentration of 50 mg/mL and subjected to five sequential passes through the ShearJet HL60 high-shear processor at 30 kpsi. Samples were analyzed using SEC-MALS before and after each pass, with data processed in ASTRA software.

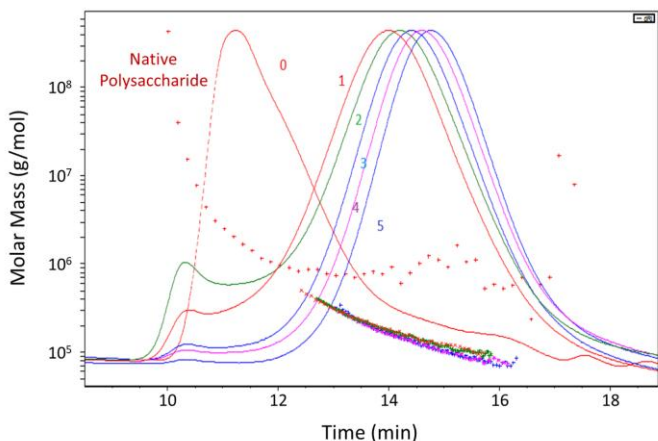


Figure 4. Light-scattering chromatograms of the native polysaccharide sample and samples taken after 1-5 passes through the ShearJet HL60, overlaid with MW from MALS.

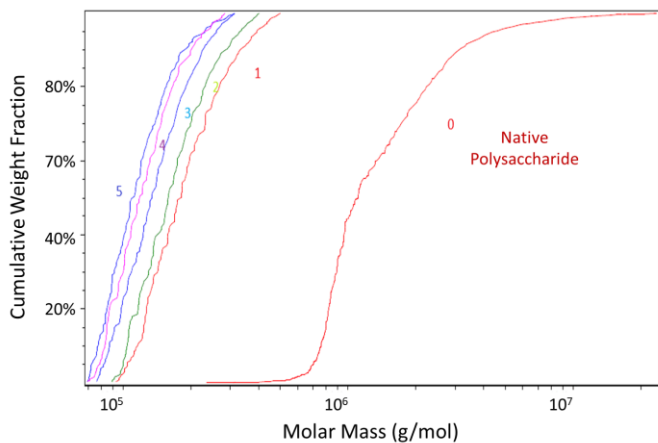


Figure 5. Cumulative weight distributions from MALS before and after processing show a steady reduction of molar mass.

After each run, the SEC-MALS chromatograms showed a clear peak shift to the right, indicating a progressive decrease in molar mass (Figure 4). In the initial measurement, the native polysaccharide eluted earlier, at a retention time of around 11 minutes, consistent with its high molar mass as confirmed by MALS. Following the

first pass through the shear jet, the peak shifted to a later retention time, reflecting the fragmentation of larger polymer chains into smaller species. This trend continued with each subsequent pass, with the peak gradually moving further to the right, confirming that the shear process systematically reduced the average molar mass of the sample.

Cumulative weight distribution analysis in ASTRA (Figure 5) confirmed the trend observed in the chromatograms, showing a reduction in molar mass with each pass. The starting material, enriched in higher molar mass species, appeared on the left side of the plot, while subsequent passes shifted the curve to the right, reflecting fragmentation into smaller chains. The steepness of the curve further reflects the polydispersity of the sample. This plot also enabled quantification of polymer fractions above or below specific molar mass thresholds, which can be useful when checking the consistency of the material between different runs or processing conditions.

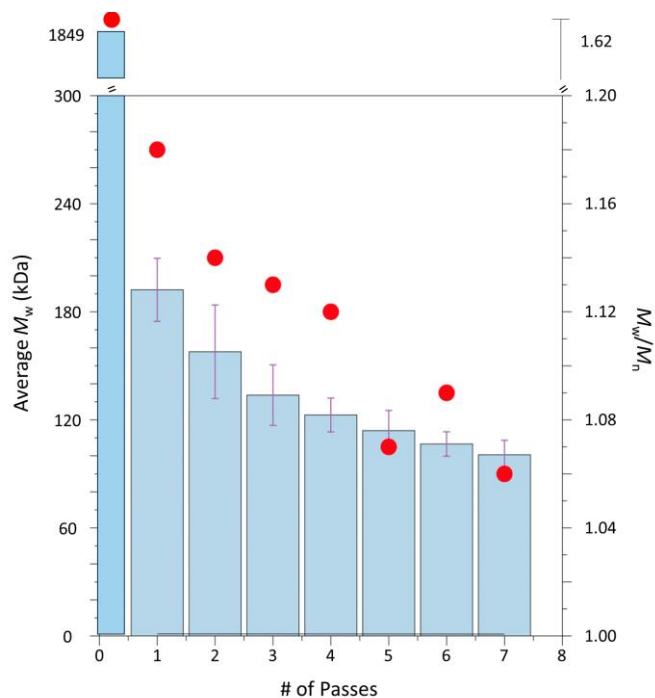


Figure 6. Reduction in both weight-averaged molar mass ( $M_w$ ) and polydispersity ( $\bullet$ ,  $M_w/M_n$ ) after each pass, showing a large decrease after the first pass followed by a steady change with additional passes.

The results of molar mass and dispersity are summarized in Figure 6, where the left y-axis represents the weight-average molar mass ( $M_w$ ) and the red dots represent the

dispersity ( $M_w/M_n$ ). Notably, a single pass through the shear jet resulted in nearly a ten-fold reduction in molar mass, as larger chains fragmented readily into smaller pieces. With additional passes, the reduction began to plateau, since progressively greater energy was required to further degrade shorter chains. Importantly, the shear jet process also reduced dispersity, yielding polymers that were increasingly uniform in size. This improvement in molecular uniformity is particularly advantageous for PCV development, as it enhances the consistency of conjugation and improves the overall reliability of the production process.

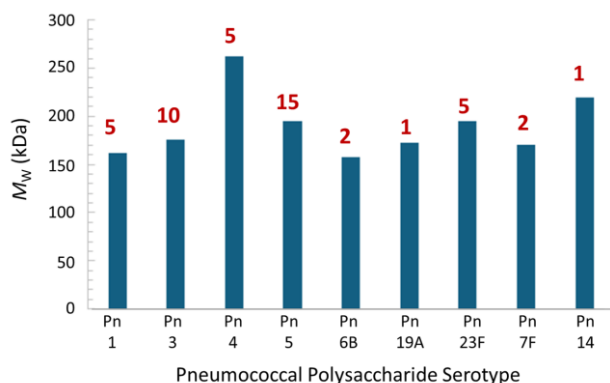


Figure 7. Shear jet processing of nine different pneumococcal polysaccharide serotypes, showing the number of passes required to reach a target  $M_w$  of 150–300 kDa.

Figure 7 shows an example study on nine different pneumococcal polysaccharide serotypes, highlighting the number of passes required to reach a target molar mass between 150 and 300 kDa. The number of passes needed depends strongly on the size and structure of the starting polysaccharide. In a typical workflow, we recommend conducting small-scale trials to characterize the initial polysaccharide and evaluate how many shear passes are necessary before scaling up to larger batch processing.

## Conclusions

Precise control of polysaccharide degradation and accurate characterization are essential for the production and consistency of conjugated vaccines. High-shear processing using the Dyhydromatics ShearJet HL60 effectively reduces both the polysaccharide's weight-average molar mass and its polydispersity, resulting in consistent drug substance quality. The use of SEC-MALS for analysis ensures that reproducible operating parameters are developed rapidly and reliably.

## About

### Fina Biosolutions

Fina Biosolutions (Rockville, MD) is a conjugate vaccine research and development company. We apply our expertise in polysaccharide conjugation to manufacture well-characterized modified dextrans. We start by offering amino dextrans with a wide range of molecular weights and amine groups, which in turn are used to build a variety of dextran reagents.

Fina Biosolutions is a tech support center for Dyhydromatics. We provide demos and conduct ShearJet method development using the Dyhydromatics HL60.

**Andrew Lees, Ph.D.**, is the founder and CEO of Fina Biosolutions, a conjugate vaccine R&D company. He has worked in the field of conjugation chemistry for over 30 years and is best known for his development of the CDAP conjugation method.

**Peter Ftacek, Ph.D.**, is the Analytical Director at Fina Biosolutions. His expertise lies in bacterial lipopolysaccharides and the development of experimental conjugate vaccines.

### Dyhydromatics

Dyhydromatics (Maynard, MA) is a leading provider of high-shear fluid processing equipment that excels in creating nanoemulsions, nanodispersions, and nanoencapsulations. The company's Shear Jet technology delivers cell disruption, and consistent particle size reduction with tight distribution for the pharmaceutical, biotech, nutraceutical, cosmeceutical, and chemical industries. With a global presence and a focus on customer support, Dyhydromatics offers comprehensive solutions for complex formulation challenges.

For more information on the technology and applications of SEC-MALS, please visit [www.wyatt.com/SEC-MALS](http://www.wyatt.com/SEC-MALS)

To request additional information, contact Wyatt Technology via [www.wyatt.com/request-info](http://www.wyatt.com/request-info).

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