WYATT TECHNOLOGY

APPLICATION NOTE

AN1306: Polyvalent pneumococcal polysaccharide vaccine characterized by SEC-MALS

Introduction

Polysaccharide molecular mass is an important parameter to monitor for potency and stability of polysaccharide-based vaccines. In fact, reduction in molecular mass of polysaccharide preparations below a threshold minimum can result in reduced immunogenicity. Therefore, molar mass is a critical quality attribute of these products that must be monitored in QC.

Multi-angle light scattering coupled to high-performance size exclusion chromatography (SEC-MALS) is an ideal method for monitoring the molecular mass of homopolymeric solutions and is utilized in development of each monovalent immunogenic polysaccharide. However,

polysaccharide vaccines typically contain mixtures of different components that have similar sizes and cannot be characterized as-is by light scattering. For QC of these materials, a different technique must be used. The secondary technique must be traceable to a highly-reliable analytical method such as SEC-MALS.

Materials and Methods

The following approach was developed to align quality control methods with release and end-expiry specifications for molecular weight of Merck's polyvalent pneumococcal polysaccharide vaccine (PNEUMOVAX®23). Each of the 23 polysaccharide components of the vaccine was separately subjected to ultrasonication to produce a series of preparations of decreasing weight-average molar mass (M_w).

These size-reduced polysaccharides were analyzed as monovalent solutions by SEC-MALS to measure their absolute molecular weight[1]. Measurements were performed with a miniDAWN® and Optilab®, while data were collected and analyzed in the ASTRA® software.

These samples were also analyzed as polyvalent formulations by high-performance size exclusion chromatography (HP-SEC) \with rate nephelometry (RN) detection to measure their relative molecular size (r-MS) based on chromatographic elution volumes[2]. The data from the two distinct molecular size analyses were used to establish a correlation between M_w and r-MS for each polysaccharide component of the vaccine. This correlation permitted the determination of each polysaccharide's r-MS end-expiry specification for the final formulated product that is in alignment with the corresponding monovalent polysaccharide M_w release specification.

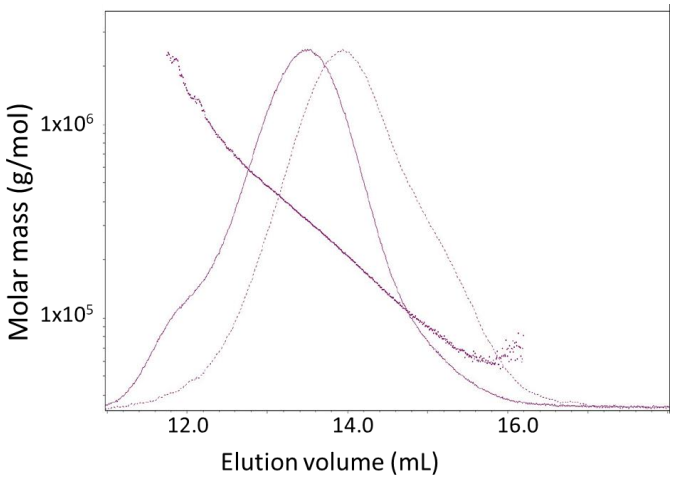


Figure 1. SEC-MALS analysis of serotype 19A polysaccharide prior to ultrasonic size reduction, M_w = 270 kDa. LS and RI chromatograms (unbroken and dashed lines, respectively) overlaid with calculated molar masses (dots).

Results and Discussion

While the entire set of results is too numerous to include here, an example is provided for one serotype polysaccharide, 19A. Figure 1 shows the SEC-MALS analysis of serotype 19A prior to ultrasonication; The molar mass distribution ranges from ~ 50 kDa to 2 MDa with a weight-average molar mass M_w of 270 kDa. After 360 minutes of sonication the lower end of the distribution did not change much but the high-molar-mass region was depleted to ~ 600 kDa, as shown in Figure 2, with a M_w value of 110 kDa.

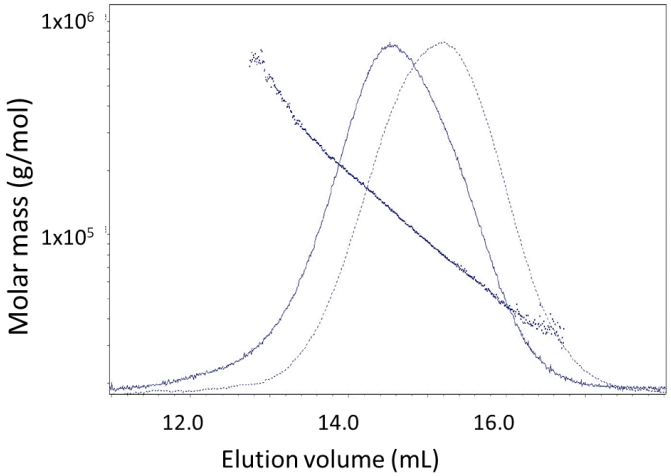


Figure 2. SEC-MALS analysis of serotype 19A polysaccharide after 360 minutes of ultrasonic size reduction, M_w = 110 kDa. LS and RI chromatograms (unbroken and dashed lines, respectively) overlaid with calculated molar masses (dots).

These and additional sonication products were subjected to r-MS analysis in order to correlate the r-MS signal with M_w determined by SEC-MALS, and the procedure repeated for each of the 23 serotypes. In this way a traceable calibration ladder was constructed for reliable QC by r-MS.

Conclusions

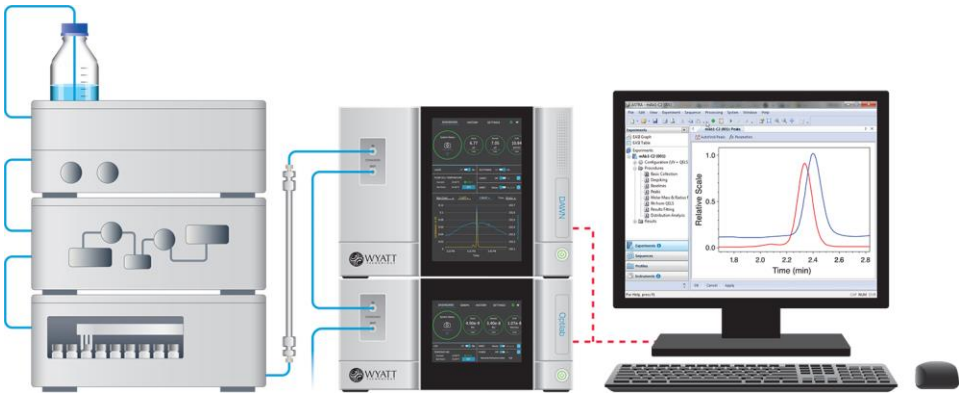
While SEC-MALS is not suitable for final-product QC of this polyvalent polysaccharide vaccine, it can be used to calibrate a secondary technique that is QC-appropriate. The alignment of specifications provides a high level of assurance that the quality control of the final vaccine product is consistent with that of the polysaccharide starting materials. This work was published[3] in Biologicals.

References

1. Bednar, B., Hennessey, J. P., Jr. (1993) "Molecular Size Analysis of Capsular Polysaccharide Preparations from Streptococcus pneumoniae" Carbohydr Res. 243, 115-130.

2. Sweeney, J. A., Sumner, J. S. and Hennessey, J. P., Jr. (2000) "Simultaneous Evaluation of Molecular Size and Antigenic Stability of PNEUMOVAX®23, a Multivalent Pneumococcal Polysaccharide Vaccine" in: Brown, Corbel, Griffiths, editors. Physico-Chemical Procedures for the Characterization of Vaccines: Dev. Biol. 103, 11-26.

3. MacNair, J., Desai, T., Teyral, J., Abeygunawardana, C., and Hennessey, J. P., Jr. (2005) "Alignment of Absolute and Relative Molecular Size Specifications for a Polyvalent Pneumococcal Polysaccharide Vaccine (PNEUMOVAX®23)" Biologicals, 33, 49-58.



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