

AN8005: RT-MALS end-point determination of a polysaccharide depolymerization process

Introduction

Molar mass plays an important role in the solubility, potency, and stability of polysaccharide-based vaccines. The production process includes a critical depolymerization step to reduce the polysaccharide's initial weight-average molar mass, M_w , from over 1800 kDa down to less than 350 kDa.

Current methods to monitor molar mass during polysaccharide production employ off-line SEC-MALS analysis, during which a single run can take up to 30 minutes. With a typical depolymerization time of about 90 minutes, off-line analytics cannot provide timely feedback on reaching the endpoint. Real-time multi-angle light scattering (RT-MALS) fills this need and ensures that the process ends as soon as the endpoint criterion is achieved.



Materials and Methods

A polysaccharide solution was depolymerized by ultrasonication at 20 kHz. Molar mass was monitored online, in near-real-time, by continuously pumping a small fraction of solution from the reactor through an **ultraDAWN™** RT-MALS instrument by means of an Agilent HPLC pump. The solution was diluted continuously by a factor of 10 in order to reduce viscosity for flow through capillary tubing. The flow rate was 5 mL/min, resulting in a lag time (RTD, residence time delay) of just 3 minutes between the time product was pulled from the reactor and time of measurement. **OBSERVER™** software was configured to control the HPLC pump, acquire data from the ultraDAWN, calculate M_w 30 times per minute, and send a trigger to stop the sonication when $M_w < 350$ kDa, as determined by the release specifications.

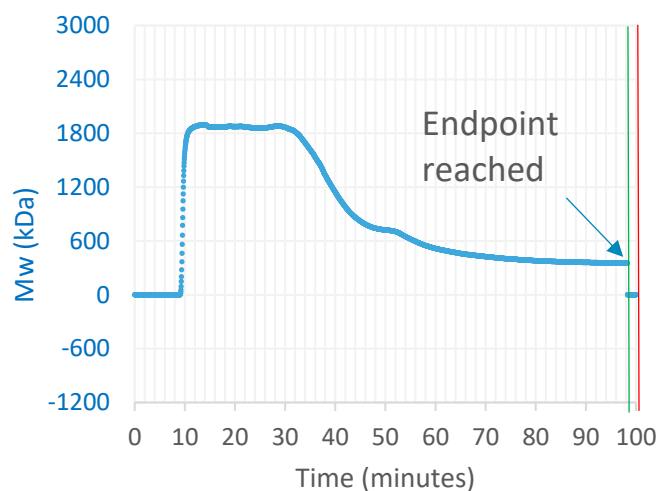


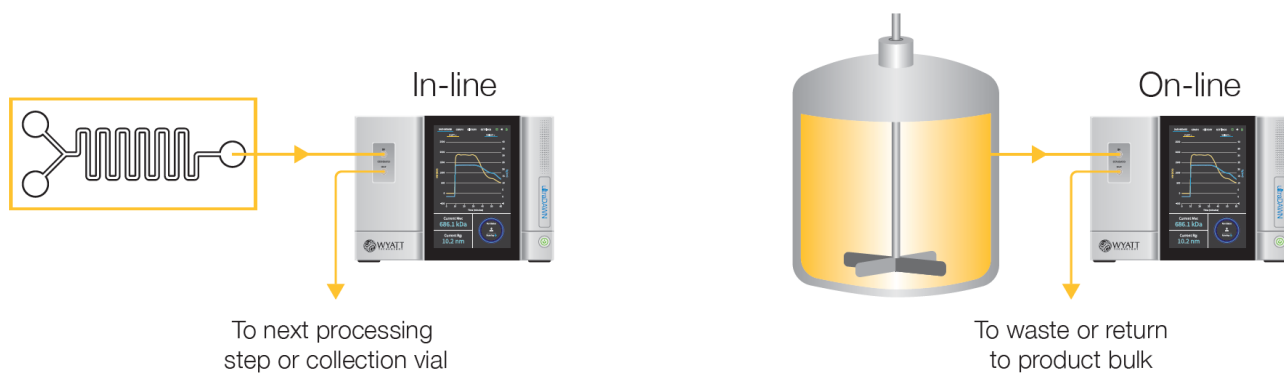
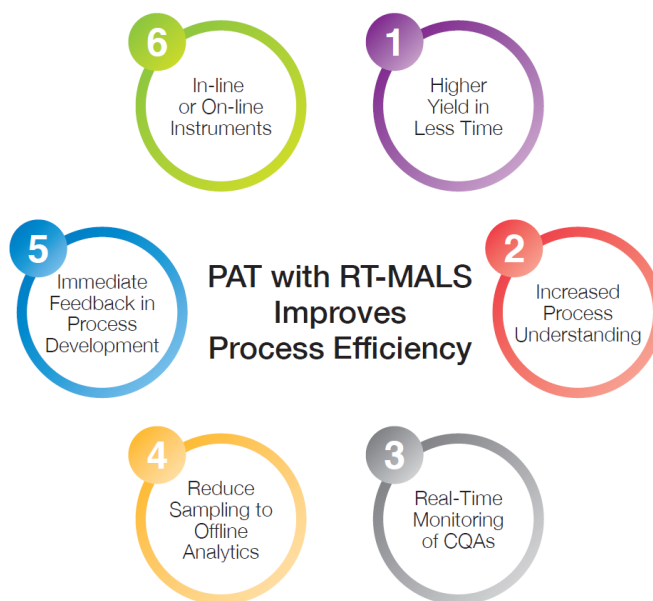
Figure 1. Trace in **OBSERVER** software of weight-average molar mass measured by ultraDAWN, indicating the achievement of the desired process endpoint.

Results and Discussion

RT-MALS demonstrably tracked reduction of the polysaccharide's M_w and triggered reaction shutdown once it fell below 350 kDa. SEC-MALS analysis of the final product confirmed the desired critical quality attribute (CQA) value. Multiple off-line analyses were eliminated, the time spent by the drug substance in this portion of the process was cut by 25%, and total person-hours spent in this part of the process was reduced by at least 50%.

Conclusions

Polysaccharide antigens are high-value-add products with substantial cost benefits to be gained by in-process determination of the primary attribute impacted by the specific process. Here, the goal of the depolymerization process is to modify the polymer's molecular weight, so the ability to determine molecular weight in near-real-time via RT-MALS reduces uncertainties due to process drift or variations in raw material properties, resulting in perfect depolymerization with every run and appreciable cost savings.



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