Application Note

Polysaccharides

Molecular weight reduction by high–shear processing on a Microfluidizer™.

Featuring two pharmaceutical case studies for conjugated vaccines containing polysaccharides

Background

Polysaccharides have been increasingly utilized in pharmaceuticals. Their diverse molecular structures and properties offer numerous advantages. They have been used, for example, as plasma expanders, carriers for water-insoluble drugs, in drug formulation, as immune stimulators and vaccine antigens. Among the most advantageous aspects is that the polysaccharide backbones are generally biocompatible and biodegradable, leading them to be naturally broken down by the body to their building blocks.

In addition to their structure, the polymer molecular weight can affect the properties of polysaccharides. Molecular weight reduction of polysaccharides has traditionally been done chemically by methods such as acid or alkaline hydrolysis and oxidation using periodates. Chemical processing can result in modification of the polysaccharide chemical structure and often results in highly polydisperse polymers.

Mechanical sizing, such as high-shear processing on a Microfluidizer™, is an alternative method of reducing the molecular weight. Microfluidization has the added benefit of reducing the molecular weight without altering the chemical structure of the polysaccharide or its attached groups. Additionally, Microfluidization generally leads to a final product that is very homogenous with low polydispersity. This is because all material that is processed on a Microfluidizer is exposed to the same high-shear conditions within the fixed geometry interaction chamber. In order to scale up processing, additional microchannels are placed in parallel to allow for increased flow rate while maintaining the equivalent high-shear processing observed at smaller volumes.

Configuration of a Microfluidizer™

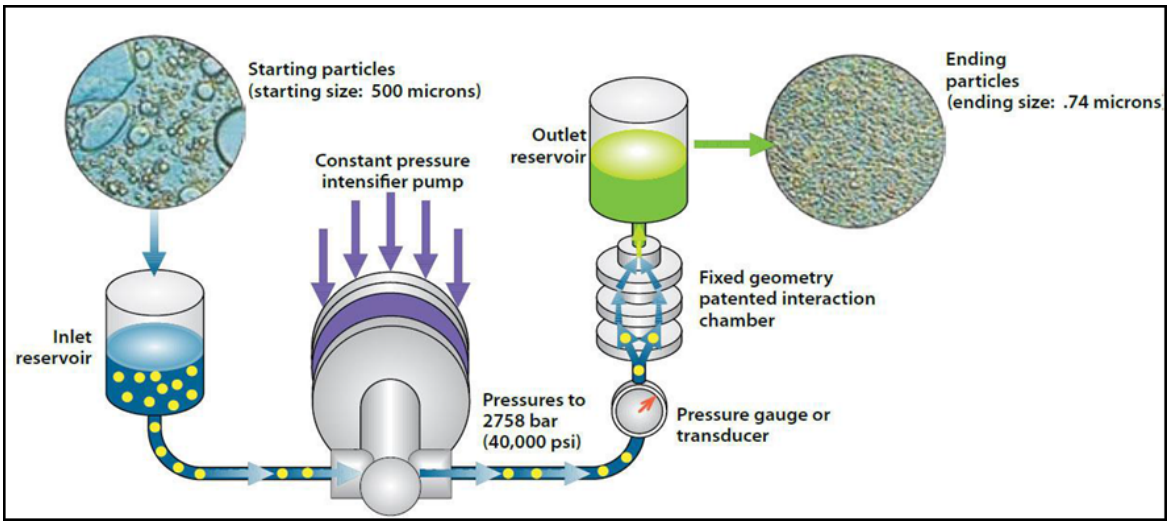

Figure 1

Featured Case Study #1:

Microfluidization for molecular weight reduction of capsular pneumococcal polysaccharides

Microfluidics has been involved in an ongoing collaborative effort with Fina Biosolutions LLC (Rockville, MD) to demonstrate the usefulness of microfluidiziation to reduce the molecular weight of polysaccharides used as vaccine antigens.

For this example, dextran, an a 1-6 glucose polymer, was used as a model. The polysaccharide molecular weight was determined by SEC HPLC on a TSKGel G4000 PWxl column (Tosoh, Tokyo, Japan) with refractive index detection using a standard curve prepared using pullulan standards (Showan Denko, Tokyo, Japan) (Figure 3). To model the molecular weight reduction of 2000 kDa dextran, JMP software (SAS, Cary, NC) was used to fit a response surface model with pressure and number of passes as the independent variables, and molecular weight as the response variable. Overlays of the chromatograms are shown in Figure 2.

Pullulan MW Standard Curve

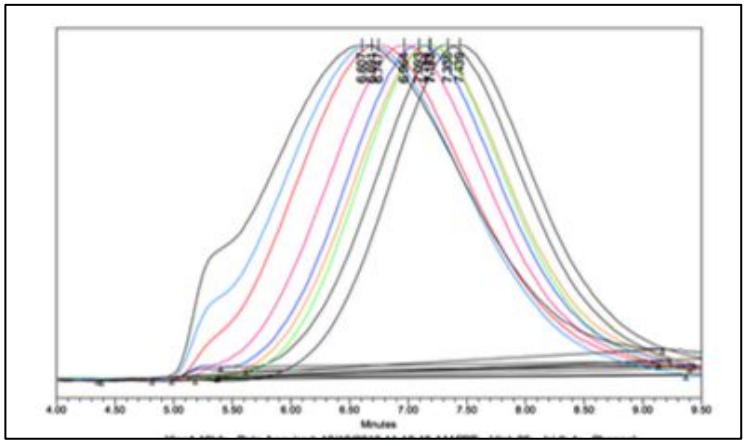

Figure 2

Microfluidized dextran

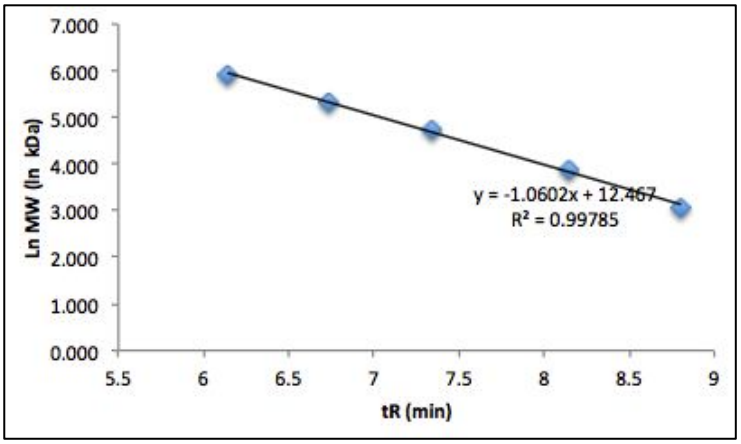

Figure 3

Predictive Test of DOE Model

A factorial experiment was used to determine the reproducibility of the Microfluidizer as well as which variables most affected molecular weight reduction. A quadratic fit of the independent variables produced a model with an $r^2 > 0.99$ with all of the coefficients being significant.

It was determined that dextran concentrations of 2-10 mg/mL were not a significant factor in reducing the polymer molecular weight (not shown).

Pressures of 14,000 psi and 17,000 psi—which were not used to develop the model—were used to test the model. The model predicted molecular weights of 200 and 178 kDa for 14k and 17k psi, respectively. The experimentally determined molecular weights were within 3% of the predicted weights, showing that the reproducibility of the Microfluidizer allows for accurate modeling.

Featured Case Study #2:

Multivalent pneumococcal conjugate vaccine

Conjugate vaccines contain proteins linked to a polysaccharide, where the polysaccharide is the bacterially derived antigen component of the vaccine. However, these polysaccharides are often very high in molecular weight. Crosslinking them to proteins leads to even higher molecular weights, creating a very viscous solution. These high molecular weights and viscosities make sterile filtration and isolation of the conjugated polysaccharide difficult. Reducing the molecular weight of the polysaccharide prior to conjugation results in a more manageable product. However, maintaining the structure of the polysaccharide, which is the antigen of interest, is critical to obtaining the proper immune response to the conjugate. Microfluidization provides an alternative to damaging chemical approaches which have traditionally been used to reduce the molecular weight.

In this example, the goal was to reduce the molecular weight of the various polysaccharide formulations to below 250 kDa using a Microfluidizer. The LV-1 was utilized due to limited material quantities.

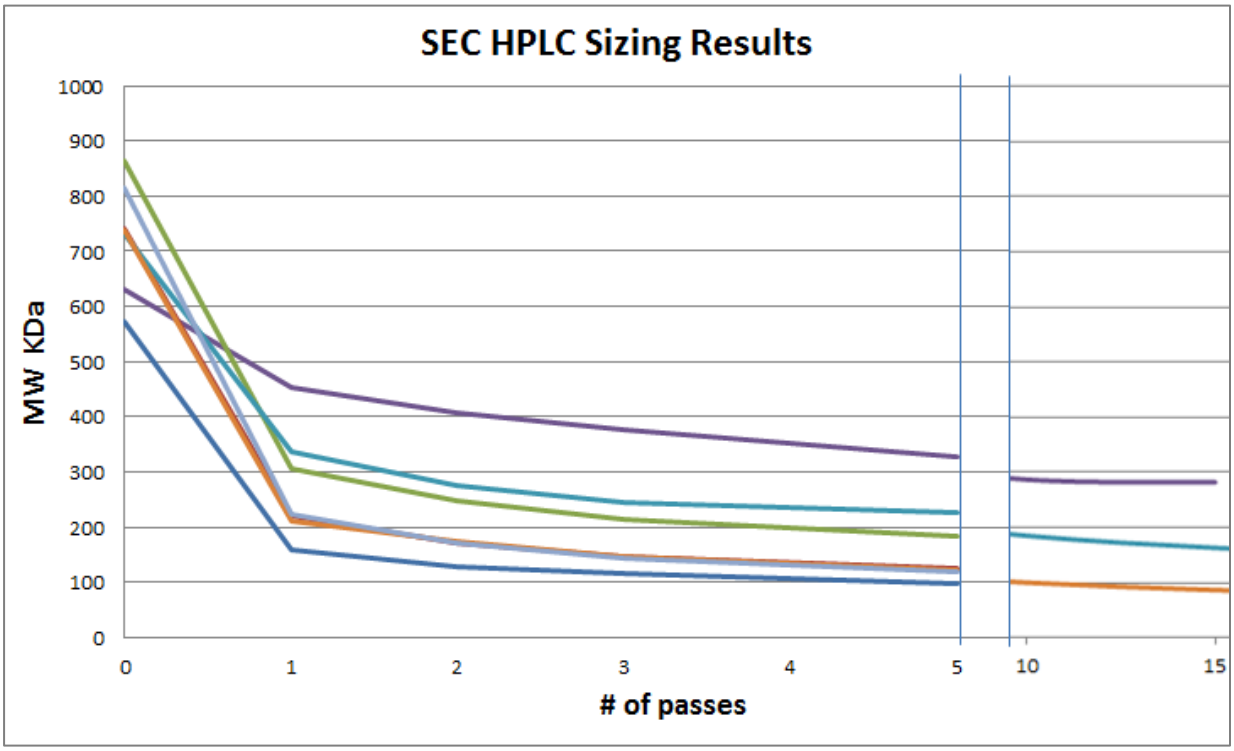

Figure 4

Conclusions

As is shown in Figure 4 above, the target molecular weights for 7 out of the 8 polysaccharides were achieved using the Microfluidizer, in some cases with a single pass. The data emphasizes that the structure of each polysaccharide is unique and the optimum number of passes must be determined for each polymer.

The LV-1 Microfluidizer is very well suited for development work with limited or expensive material due to its low volume requirements and ease of use. The Microfluidizer provides a simple, controllable, and reproducible method for determining optimum parameters for polymer molecular weight reduction. Furthermore, the same parameters can be used to directly scale-up the process.

Notes and References

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