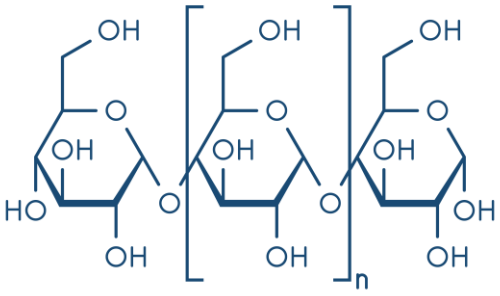
Microfluidics™

Application Note

Microfluidizer® Technology for Polysaccharides



INTRODUCTION

Polysaccharides are being increasingly used in pharmaceuticals. Their diverse molecular structures and properties mean they have been used 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 & biodegradable, leading them to be naturally broken down by the body to their building blocks. In addition, physiochemical properties such as molecular weight are found to have significant impact on the bioactivity of polysaccharides. Higher molecular weight makes the penetration of polysaccharides through cell membrane barriers more difficult, which reduces their pharmacological effects. Reducing molecular weight helps to improve physical properties such as viscosity to facilitate downstream purification processes as well as making sterile filtration possible.

This paper looks at two case studies that study conjugated vaccines containing polysaccharides.

Microfluidizer® Technology for Polysaccharides

MICROFLUIDIZER PROCESSOR ADVANTAGES

Traditionally, molecular weight reduction of polysaccharides has been done chemically by methods such as acid or alkaline hydrolysis and oxidation using periodates.

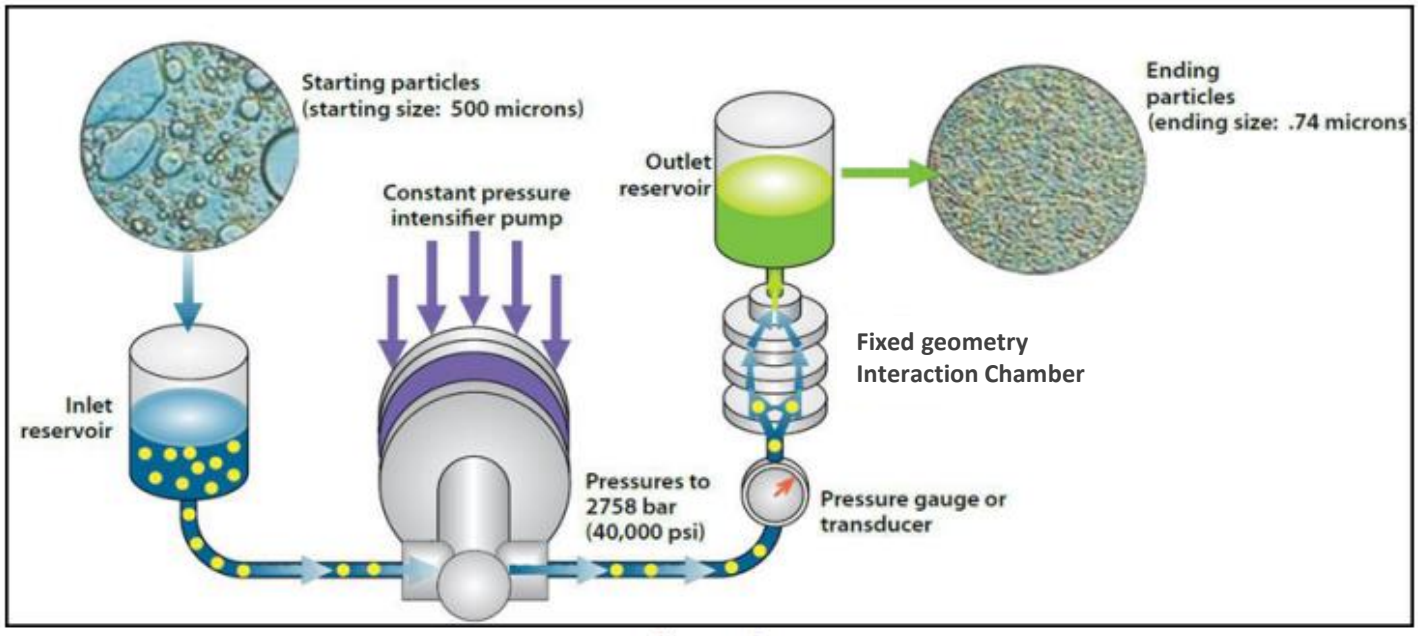
Chemical processing can result in undesired modification of the polysaccharide chemical structure and often results in highly polydisperse polymers.

Mechanical sizing, such as high-shear processing on a Microfluidizer® processor, is an alternative method for reducing the molecular weight. Processing through a Microfluidizer® processor has the added benefit of reducing the molecular weight without altering the chemical structure of the polysaccharide or its attached groups.

Additionally, homogenization achieved via Microfluidizer® technology will generally lead to a final product that is very homogenous with low polydispersity.

This is because all material that is processed on a Microfluidizer® processor is exposed to the same high-shear conditions within the fixed geometry Interaction Chamber™.

In order to scale up the process, additional microchannels are placed in parallel inside the Interaction Chamber™ to allow for increased flow rate while maintaining the equivalent high-shear processing observed at smaller volumes.



Microfluidizer® Technology for Polysaccharides

CASE STUDY #1

Molecular weight reduction of capsular pneumococcal polysaccharides

Microfluidics has been involved in an ongoing collaborative effort with Fina Biosolutions LLC (Rockville, MD) in demonstrating the effectiveness of Microfluidizer® technology in reducing 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 2).

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 3.

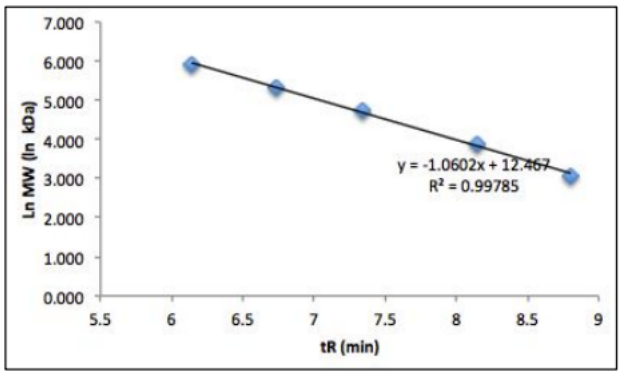


Figure 2 – Dextran processed with Microfluidizer® processor

PREDICTIVE TEST OF DOE MODEL

A factorial experiment was used to determine the reproducibility of the Microfluidizer® processor as well as deciding which variables mostly 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 (data 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 values, showing that the reproducibility of the Microfluidizer® processor allows for accurate modeling and subsequently achieving precise process controls.

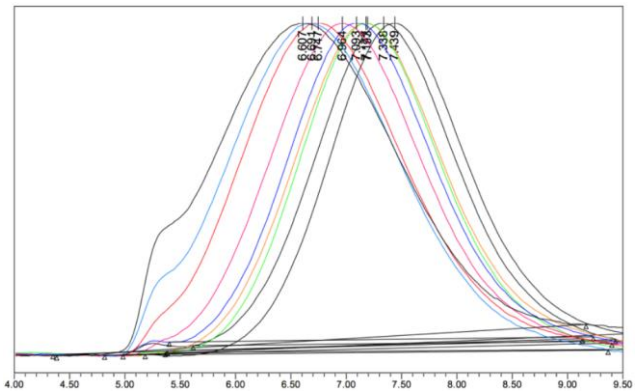


Figure 3 – Pullulan MW Standard Curve

Microfluidizer® Technology for Polysaccharides

CASE STUDY #2

Multivalent pneumococcal conjugate vaccine

In this case study a conjugate vaccine was formed by cross-linking proteins to bacterial polysaccharides, where the polysaccharides serve as the antigen component of the vaccine. However, due to the high molecular weights of these polysaccharides cross-linking them to proteins leads to even higher molecular weights and creates 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 polysaccharides prior to conjugation results in a 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.

Microfluidizer® technology provides an alternative to damaging chemical approaches which have traditionally been used to reduce the molecular weight. The aim was to reduce the molecular weight of the polysaccharide formulations to below 250 kDa using a Microfluidizer Processor. The LV1 Microfluidizer processor was utilized due to limited material quantities.

RESULTS

As is shown in Figure 4, the target molecular weights for 7 out of the 8 polysaccharides were achieved using the Microfluidizer® processor, in some cases with only 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 polysaccharide.

The LV1 Microfluidizer® processor is very well suited for development work with limited or expensive material due to its low volume requirements and ease of use. The process conditions and results can be directly scaled up to any of the Microfludics larger benchtop units as well as larger scale machines.

This case study has demonstrated that Microfluidizer® technology provides a simple, controllable, and reproducible method for determining optimum parameters for polysaccharide molecular weight reduction.

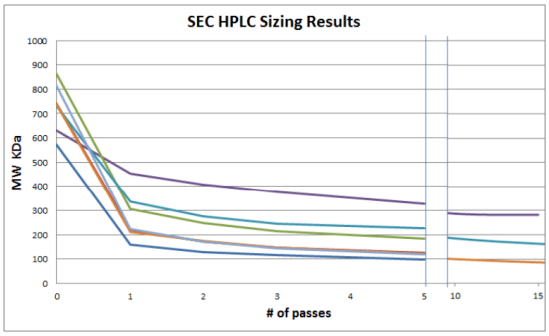


Figure 4 – Comparison of various polysaccharides processed with a Microfluidizer® processor