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Impact of module geometry on the ultrafiltration behavior of capsular polysaccharides for vaccines

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ABSTRACT

Ultrafiltration is an important step in the downstream processing of polysaccharide-based vaccines. The objective of this paper was to examine the ultrafiltration behavior of several native polysaccharide serotypes (Pn3, Pn9V, and Pn14) in different membrane modules. The transmission of Pn9V and Pn14 are governed by concentration polarization effects, with the greatest extent of polarization seen in the stirred cell followed by the hollow fiber module and then the tangential flow filtration (TFF) cassette as expected. In contrast, transmission of the highly charged Pn3 serotype in the hollow fiber and TFF cassette reached a maximum value of only about 20% due to membrane fouling, even though data in the stirred cell showed no effects of fouling on the sieving coefficient under otherwise identical conditions. The high degree of fouling by Pn3 appears to be associated with elongation of this high molecular weight polysaccharide in the high shear environment in the tangential flow modules. Rheological data for Pn3, which was the most highly charged and largest molecular weight polysaccharide examined in this work, showed a high degree of shear-thinning. These results provide important insights into the effects of polarization, fouling, and module design on the ultrafiltration behavior of these important polysaccharides.

1. Introduction

There are significant opportunities for using ultrafiltration in the downstream processing of capsular polysaccharides and polysaccharide-protein conjugates used as vaccines against pneumococci and meningococci [1–3]. This includes concentration and buffer exchange, as well as the removal of residual free polysaccharides from the desired polysaccharide-protein conjugate formed via an appropriate coupling reaction. These conjugated vaccines provide much better immunization, particularly in young children and the elderly. The removal of free polysaccharides requires the use of large pore size ultrafiltration membranes or even small pore size microfiltration membranes given the high molecular weight of the free polysaccharides (from several hundred to several thousand kDa).

Previous studies of polysaccharide ultrafiltration have been performed in a number of different types of membrane modules. For example, Wen et al. [2] used hollow fiber membranes with 0.05 and 0.1 μm pore size for the removal of unreacted bacterial polysaccharides from a conjugate formed by the coupling of these polysaccharides to the outer membrane protein complex from a *Meningitidis* bacteria. Goncalves *et al.* [4] used tangential flow filtration (TFF) cassettes with flat-sheet membranes and a spacer-filled channel for the purification of polysaccharide serotype 23 F from *Streptococcus pneumoniae*. Takagi et al. [5] used a spiral wound membrane module containing flat sheet 100 kDa molecular weight cut-off membranes to remove small protein impurities from a capsular polysaccharide produced by *Haemophilus influenzae*. Zanardo et al. [6] used a combination of TFF cassettes and a spiral wound module for the purification of polysaccharide serotype Pn14 from *Streptococcus pneumoniae*. More recently, Hadidi et al. [3] used a stirred ultrafiltration cell to study the retention characteristics of several pneumococcus polysaccharide serotypes (obtained from distinct strains of the microorganism) using large pore size flat sheet ultrafiltration membranes with molecular weight cut-offs between 100 and 1000 kDa.

It is very difficult to quantitatively compare results from these experimental studies due to differences in the polysaccharides, the membranes, and just as importantly the modules (stirred cell, hollow fiber, TFF cassette, and spiral wound). There have been a number of previous studies that have rigorously compared the ultrafiltration behavior of different modules using well-defined model systems. For

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example, Zydney and Xenopoulos [7] evaluated the ultrafiltration behavior of polydisperse dextrans in both a stirred cell and Minitan device (open channel TFF module) using membranes with molecular weight cutoffs between 5 and 300 kDa. The mass transfer coefficient in the Minitan device was somewhat smaller than that in the stirred cell due to the absence of any spacer element. More significantly, there was a large variation in filtrate flux with position in the TFF module due to the pressure variation associated with flow through the module. This also led to back-filtration at low filtrate flux arising from the negative transmembrane pressure near the device exit. The net result was that the dextran retention curves were less reproducible, and more difficult to interpret, in the Minitan device, particularly for larger molecular weight dextrans.

Dang et al. [8] compared the performance of a stirred cell and two tangential flow filtration modules, a commercial module with internal screen and a self-designed module with open channel, during a series of fouling / cleaning experiments using raw water containing high concentrations of natural organic matter (NOM). The self-designed module had the highest initial flux, which the authors attributed to the high shear rates in the very thin channel. The stirred cell provided the lowest level of NOM removal, which may have been due to the high degree of concentration polarization in this system. On the other hand, the membrane used in the stirred cell was most effectively cleaned, although this required removal of the membrane from the module so that it could be effectively backwashed.

The objective of this study was to provide a quantitative comparison of the ultrafiltration behavior of three native capsular polysaccharides currently of interest in vaccine development using a stirred cell, TFF cassette, and hollow fiber membrane module with large molecular weight cutoff ultrafiltration membranes. Note that the actual polysaccharides used to generate conjugate vaccines are treated prior to use and are therefore considerably smaller than the native polysaccharides examined in this work. The stirred cell and TFF cassettes used the same membrane (Biomax® polyethersulfone with 500 kDa nominal molecular weight cut-off); the hollow fiber module also used a polyethersulfone membrane with the same nominal molecular weight cutoff but from a different manufacturer. Experiments were also performed using dextrans with similar size to the polysaccharides. Data were analyzed using the classical stagnant film model for concentration polarization with the appropriate mass transfer coefficients in the different modules. The results demonstrate the importance of both mass transfer and fouling on the overall polysaccharide transmission, with the relative importance of these phenomena depending on the properties of both the polysaccharides and the membrane modules.

2. Experimental

2.1. Solution preparation

Buffer solutions were prepared using Bis–Tris (MP Biomedical, 101038) with added KCl (BDH Chemicals, BDH0258). The pH was adjusted to 7.0 using KOH or HCl as needed. All buffers were prefiltered through 0.2 μm pore size membranes (Supor® 200, Pall Corporation) before use.

Native capsular polysaccharides of the *Streptococcus pneumoniae* bacteria were provided by Pfizer Inc. (Chesterfield, MO). Three different serotypes were examined having a range of molecular weight and charge density. Pn3 and Pn9V are both negatively charged due to the presence of glucuronic acid in the repeating structure, with Pn3 having the higher charge density and the greater molecular weight. Pn14 is composed of only neutral monosaccharides.

Different molecular weight dextrans were obtained from American Polymer Standards Corp. (Mentor, OH). Dextrans with equivalent hydrodynamic radius as the bacterial polysaccharides were chosen based on equal retention volumes in size exclusion chromatography as determined using a PL Aquagel-OH 60 size exclusion column (Agilent Technologies, CA).

Table 1
Properties of the stirred cell, hollow fiber, and TFF cassette modules. Parameters a, b, c, and β describe the mass transfer coefficient in the module based on Eq. (8).

Membrane	*Stirred cell*	*Hollow fiber*	*TFF Cassette*
Material	Polyethersulfone Biomax®	Polyethersulfone	Polyethersulfone Biomax®
Area (cm^2)	4.1	28	50
MWCO (kDa)	500	500	500
Dimensions (mm)	25 (cell diameter)	0.5 (fiber diameter) 200 (fiber length)	30 (channel width) 188 (channel length) 0.53 (channel height)
a	0.567	0.33	0.5
b	0.33	0.33	0.33
c	-	0.33	0.5
β	0.23	1.62	0.664

2.2. Membranes / Modules

Ultrafiltration experiments were performed using an Amicon 8010 stirred cell (Millipore Corporation, MA), MicroKros® hollow fibers (Spectrum Labs, Carlsbad, CA), and Pellicon XL TFF cassettes (Millipore Corporation, MA). The stirred cell and TFF cassette both used Biomax® polyethersulfone 500 kDa molecular weight cut-off membranes; the hollow fiber membrane was also polyethersulfone with the same nominal molecular weight cutoff but from Spectrum Laboratories. Physical properties of these membranes / modules are summarized in Table 1.

Stirred cell experiments were performed with membrane disks cut from a large flat sheet and then soaked in 90% isopropanol for 45 min. The membrane was then rinsed with deionized (DI) water, placed in the base of the stirred cell, and sealed with an O-ring. The stirred cell was filled with DI water and connected to an acrylic solution reservoir pressurized by nitrogen. The stirring speed was set to 1200 rpm using a digital stroboscope (Extech Instruments, Nashua, NH), the membrane was flushed with approximately 100 L/m^2 of DI water, and the hydraulic permeability evaluated as described below. Membranes were discarded after each experiment.

The MicroKros® hollow fiber module was used with flow in the into-out configuration. The modules were initially washed with 0.1 N NaOH at 40 °C for 20 min following the manufacturer's recommendation and then flushed with 2 L/m^2 of DI water before evaluating the permeability. After each experiment, the hollow fiber module was cleaned with 0.1 N NaOH at 40 °C with periodic backflushing (flow from out-to-in). The modules were stored wet, filled with 0.1 N NaOH, at 4 °C.

Pellicon XL TFF cassettes were initially washed with 0.5 N NaOH at 40–45 °C for 45–60 min followed by 2 L of DI water. The cassettes were cleaned using the same procedure immediately after each experiment, with the module stored in 0.1 N NaOH at 4 °C.

2.3. Ultrafiltration experiments

Ultrafiltration experiments were conducted at room temperature (22 ± 2 °C). The membrane hydraulic permeability (L_p) was first evaluated from data for the buffer flux (J_v) at several transmembrane pressures (TMP):

$$L_p = \mu \frac{J_v}{TMP} \quad (1)$$

where μ is the viscosity of the buffer solution (assumed to be equal to that of water at the same temperature). The transmembrane pressure in