Optimization of High Antibody Concentration Using Hollow Fiber Tangential Flow Filtration (HF TFF)

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Background/Introduction

Antibodies (Abs), or immunoglobulins (Ig), have been widely used in a number of research and therapeutic applications.[1] Use of monoclonal antibodies (mAbs), in particular, has dramatically increased in diagnostics, protein purification, and medical applications.[2-5] A major hurdle for the use of therapeutic mAbs is that high concentrations of antibodies are needed for subcutaneous injection, the preferred method of delivery.[6] As required by the FDA, administered volumes need to be less than 1.5 ml when applied subcutaneously, meaning that formulations may need to be prepared at concentrations greater than 100 mg/ml.[7] However, such high concentrations of mAb formulations often present manufacturing challenges due to issues with stability, solubility, high viscosity, and aggregation.[8,9] Similar issues arise with Ab formulations as well.

Hollow fiber tangential flow filtration (HF TFF) is a superior method for the high concentration of Abs compared to cassette filters because the non-turbulent flow dynamics within the fibers provide significantly lower pressure drops. For most high concentration Ab applications using cassette TFF filters, the feed pump needs to be slowed down to maintain constant pressure as the viscosity of the antibody increases. In HF TFF, feed pumps do not need to be controlled because the pressure buildup is minimal. Additionally, the recovery and drainage of products using HF filters is simplified due to a lack of turbulence promotors or screens that are seen in cassette filters. Finally, the option of single-use HF filter modules eliminates extensive cleaning, assembly, disassembly, and maintenance procedures, ultimately saving time and overhead costs that are commonly associated with traditional cassette filters.

This application note examines the use of HF membranes for the efficient concentration of IgG, and the effect of shear rates and transmembrane pressure (TMP) on permeate flux rates.

Materials and Methods

System: KR2*i* TFF System
(SYR2U20,SpectrumLabs.com)
Filters: 30 kDa mPES, MicroKros® (C02-E030-05-N, 0.5 mm; C02-E030-10-N, 1.0 mm, SpectrumLabs.com)

Preparation of Samples

IgG was obtained from commercial sources (Sigma Aldrich) and was dissolved in a 0.9% saline solution to a final concentration of 30 mg/ml. The solution was filtered through a 0.2 µm filter to remove larger particles prior to running experiments.

Initial and final concentrations of IgG were determined by measuring the absorbance at 280 nm, and using Beer's Law to calculate concentration. ε_{280} = 210,000 M^{-1} cm^{-1}.

Preparation of Filter Modules

The filter modules were wetted and tested for integrity prior to concentration. MicroKros filter modules with a molecular weight cut-off (MWCO) of 30 kDa, containing modified polyethersulfone (mPES) fibers with inner diameters (ID) of either 0.5 mm (6 fibers per module, 20 cm^2) or 1.0 mm (2 fibers per module, 13 cm^2), were utilized for experiments conducted in this application note. The wetting and integrity test were performed using a KR2*i* TFF system. Briefly, the filter modules were flushed with DI water until a permeate volume of 2 ml per cm^2 of surface area was reached. To test the fiber and module integrity, a leak test was performed. The module was fully wetted out and the extra-capillary space (ECS) was flooded with water. The retentate was blocked and air was slowly introduced into the module through the inlet. The pump was stopped after the transmembrane pressure (TMP) reached ~5 psi. A lack of bubbles in the ECS and a constant feed pressure indicated an integral filter module. After the integrity check, 0.9% saline solution was run through the filter until a permeate volume of 2 ml per cm^2 was collected.

Concentration Trials

Concentration experiments were conducted using a KR2*i* TFF system. Trial runs were conducted at a shear rate of 6,000 s^{-1} for filter modules containing both 0.5 and 1.0 mm inner diameter HF membranes. Data (flow rates, pressures, etc.) was collected using the integrated KF Comm software. The IgG solution (30 mg/ml) was added to a conical reservoir tube (3 port w/ dip tubes, SpectrumLabs.com). The permeate line was directed to a collection vessel. The progress of the concentration was monitored by measuring the mass of collected permeate, which is automatically detected by the KF Comm software. The protein solution was introduced into the module by slowly ramping to the desired flow rate (6,000 s^{-1} shear rate) over 3 seconds. The KR2*i* system was set to Concentration (C) mode.

Shear Rate Trials

To test the effect of different shear rates on permeate flux, an IgG solution (~100 mg/ml) was recirculated through HF membranes. To ensure a constant concentration of IgG throughout the process, the solution of IgG was continuously diafiltered with 0.9% saline solution. The set-up was identical to that of C mode, except that the 3rd dip tube from the conical tube was connected to an auxiliary reservoir filled with buffer, rather than open to air. This flow path allows for continuous addition of buffer to the IgG solution at a rate identical to the permeate flux via the vacuum formed during processing. Shear rate experiments were conducted at flow rates that provided the appropriate shear rate, and with no applied backpressure. Experiments were conducted on filter modules containing both 0.5 and 1.0 mm inner diameter HF membranes, and data was collected using the KF Comm software.

Transmembrane Pressure (TMP) Trials

The effect of transmembrane pressures (TMP) on filter performance was examined by applying backpressure onto the retentate line at constant shear rates. Experiments were conducted using MicroKros filter modules containing 0.5 and 1.0 mm inner diameter HF membranes. Shear rates of 6,000 and 10,000 s^{-1} were utilized for this experiments. The experiments were set-up in a similar manner to that of the shear rate experiments, with one notable difference. A secondary diafiltration pump was utilized to continuously add buffer (0.9% saline) to the protein solution in concentration/diafiltration (C/D) mode to maintain an IgG concentration of 30 mg/ml throughout the experiment (the concentration factor was set to 1 so diafiltration began immediately). Data was collected using the KF Comm software.

Results and Discussion

Concentration Trial (0.5 mm inner diameter)

For an initial experiment, we tested the maximum limit to which IgG could be concentrated to using HF membranes. A MicroKros module containing 0.5 mm inner diameter fibers was used. In this experiment, 20 ml of the 30 mg/ml solution of IgG in 0.9% saline was placed into a 50 ml conical processing reservoir with a 3 dip-tube cap. Two dip-tubes were connected to the inlet and retentate tubing to and from the module, respectively, while the third dip tube was open to air.

The IgG solution was slowly introduced into the tubing and module with the permeate line closed. Once the recirculation flow rate was constant, the permeate line was opened, and concentration began. In this experiment, there was no external backpressure added (via the retentate line), so the TMP was left to increase as the viscosity of the solution increased with concentration. The inlet flow rate 27 ml/min (6,000 s^{-1} shear rate) was also left constant.

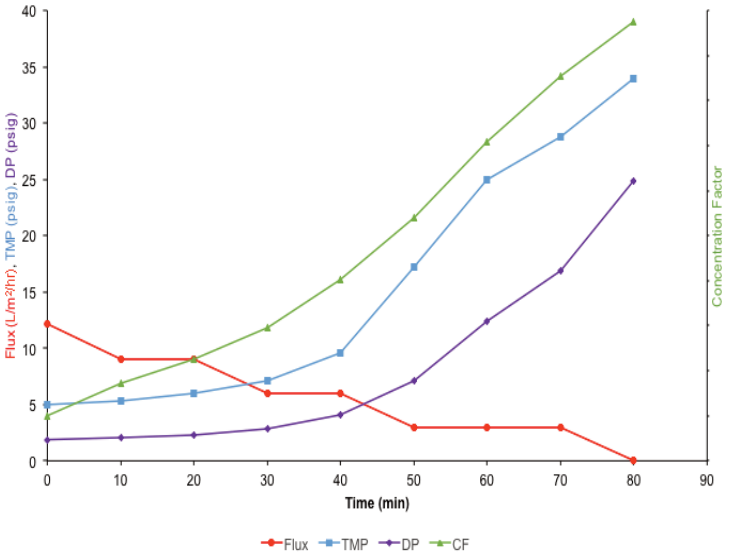

Figure 1 Flux, TMP, pressure drop (DP), and concentration factor (CF) data from high concentration run using MicroKros module containing 0.5 mm ID mPES HF membranes.

As shown in Figure 1, the TMP immediately increased as soon as concentration began. Concomitantly, the flux

began to decrease steadily. Pressure drop (DP) across the module is indicative of filter fouling, and often plagues processes that involve concentration steps. However, Figure 1 showed that the pressure drop was maintained under 10 psig, even after 60 minutes of processing. After 60 minutes, the pressure drop increased past 10 psig, eventually reaching approximately 25 psig and the flux dropped to 0 L/m^2/hr.

The final concentration of the IgG solution was determined by UV-spectrophotometry and was found to be approximately 350 mg/ml, corresponding to a ~12x increase in concentration.

Concentration Trial (1.0 mm inner diameter)

We also ran a concentration experiment using filter modules containing HF membranes containing 1.0 mm inner diameter fibers. A shear rate of 6,000 s^{-1} (71 ml/min) was held constant for this process. The TMP was controlled via the automatic backpressure valve, and was kept to 5 psig. The results are shown in Figure 2.

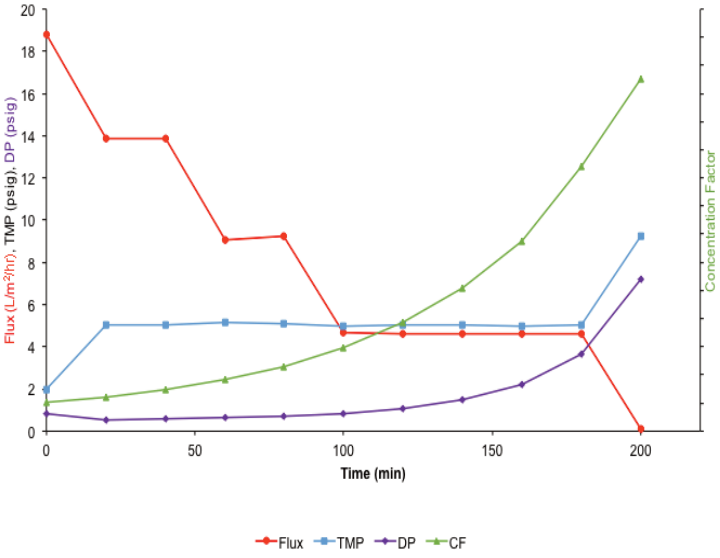

Figure 2 Flux, TMP, DP, and CF data from concentration run using MicroKros module containing 1.0 mm ID mPES HF membranes. TMP was held constant at 5 psig for duration of run (except for the last data point)

As shown in Figure 2, the initial flux of the 1.0 mm inner dimeter fibers was greater than that of those with 0.5 mm inner diameter fibers (18 L/m^2/hr vs 12 L/m^2/hr, respectively). As predicted, the flux decreased as the concentration of the IgG solution increased. During the run, the TMP stayed constant at 5 psig, until the very end of the run when it increased to approximately 10 psig. It was also at this time that the flux dropped to 0 L/m^2/hr. Figure 2 also shows an overall pressure drop of less than 8 psig throughout the run, suggesting low membrane fouling. This shows that the 1.0 mm fibers allowed for better filter flux during the concentration process.

The concentration of the final IgG solution was measured by UV-spectrophotometry, and was calculated to be approximately 226 mg/ml, a 7.5-fold concentration. Use of the 1.0 mm inner diameter fibers required larger tubing to reach the appropriate flow rate of 71 ml/min. Due to the increase in hold-up volume, concentrations greater than 250 mg/ml could not be obtained. However, under optimal conditions, higher concentrations could be reached.

Shear Rate Trials

Due to the lower pressure drops shown by HF membranes for the concentration of IgG, increased feed flow/shear rates can be utilized, shortening processing times. To test the effect of shear rates on filtrate flow rates, a solution of IgG (~100 mg/ml) was recirculated at increasing feed flow/shear rates. Experiments were run with no added back pressure.

Increasing the shear rates showed increased filtrate flux for both the 0.5 (Figure 3) and 1.0 mm (Figure 4) fibers. As shown in Figure 3, filtrate flux increased linearly with increasing shear rates, climbing from 6 L/m^2/hr to 10 L/m^2/hr from 4,000 s^{-1} to 12,000 s^{-1}, respectively. However, it should be noted that even without applied backpressure, the minimal TMP was still 5 psig at the lowest shear rate (4,000 s-1). When increased to 12,000 s^{-1}, the TMP increased to approximately 20 psig.

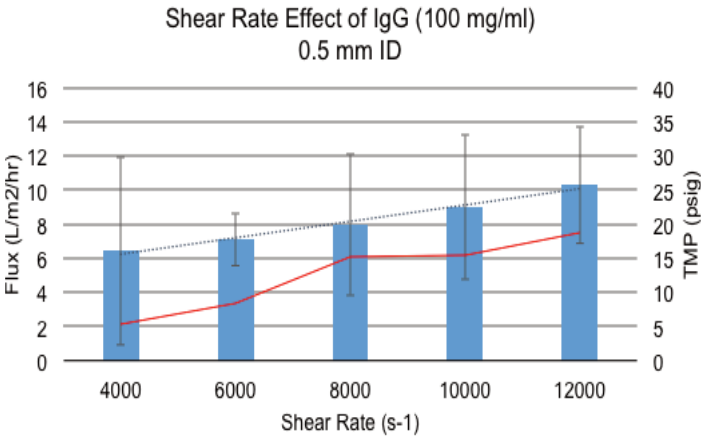

Figure 3 Effect of increasing shear rates on filtrate flux for 0.5 mm ID HF membranes

The filter modules containing 1.0 mm inner diameter HF membranes displayed a significant increase in filter flux, as well as a large decrease in transmembrane pressure compared to that from 0.5 mm inner diameter HF membranes. Figure 4 shows that filter flux increases 4-fold, from 4 L/m^2/hr to 16 L/m^2/hr. Importantly, transmembrane pressure essentially remains unchanged, and never increases past 5 psig, even at a shear rate of 11,000 s^{-1}. As a comparison, at a TMP of 5 psig, the 0.5 mm inner dimeter fibers were only able to exhibit a filter flux of approximately 6 L/m^2/hr.

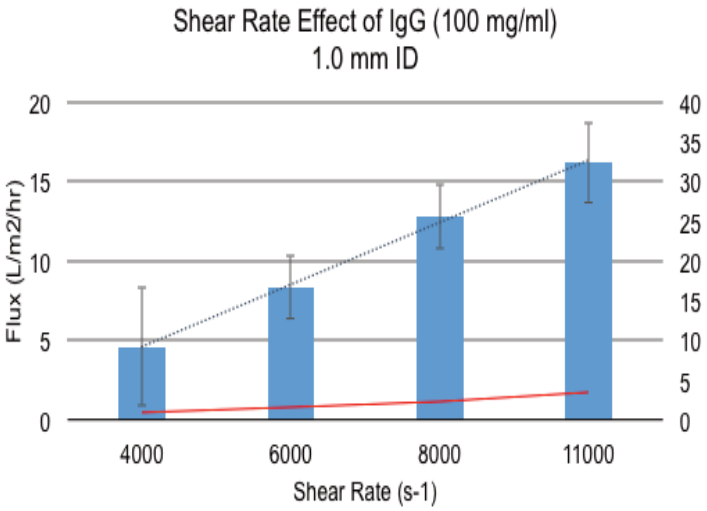

Figure 4 Effect of increasing shear rates on filtrate flux for 1.0 mm ID HF membranes

TMP Trials

In our final experiment, we tested the effect of added backpressure on filter performance at a given shear rate. In this experiment, filter modules containing 0.5 and 1.0 mm inner diameter fibers were tested at shear rates of 6,000 and 10,000 s^{-1}, while the TMPs were increased from 5 to 20 psig. The results are shown in Figure 5 and Figure 6. The experiments were conducted using an IgG solution of 30 mg/ml in 0.9% saline.

As shown in Figure 5, increasing the backpressure had a negative effect on filter flux, as it decreases from 14 L/m^2/hr to 9 L/m^2/hr when the shear rate was 6,000 s^{-1} for filter modules containing 0.5 mm inner diameter fibers. This suggests that added backpressure enhances the formation of a gel layer, ultimately slowing filter performance. Interestingly, when the shear rate was increased to 10,000 s^{-1} and the TMP returned to 5 psig, the filter flux increased to 18 L/m^2/hr, suggesting that the increased flow rate is capable of sweeping and dislodging the settled IgG from the membrane surface. Furthermore, increasing the TMP to 10, 15, and 20 psig did not show a detrimental effect to filter flux, providing evidence that the increased shear rate sweeps the membrane surface effectively and continuously at 10,000 s^{-1}. In turn, this sweeping effect impedes gel layer formation, even at high TMPs.

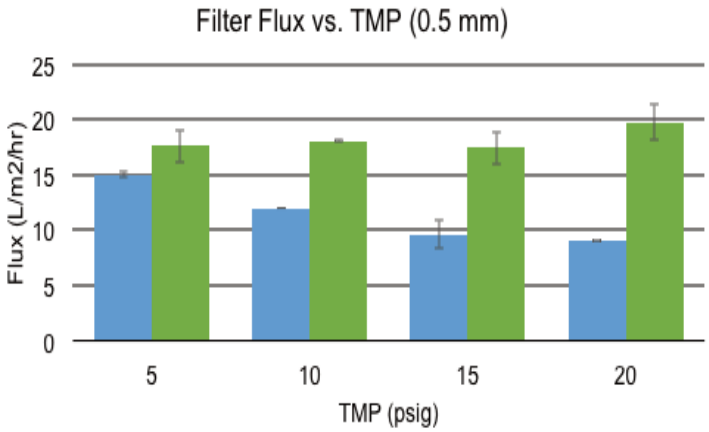

Figure 5 Results of increasing TMP via backpressure on 0.5 mm inner diameter HF membranes

Figure 6 shows the results from the identical experiment using filter modules containing 1.0 mm inner diameter fibers. As shown, the filter fluxes at shear rates of 6,000 or 10,000 s^{-1} do not decrease when TMP is increased. The flux was maintained at approximately 15 L/m^2/hr at 6,000 s^{-1} at all TMPs tested. The filter flux at 10,000 s^{-1} slightly improved from 20 to 23 L/m2/hr when the TMP was increased from 5 to 20 psig.

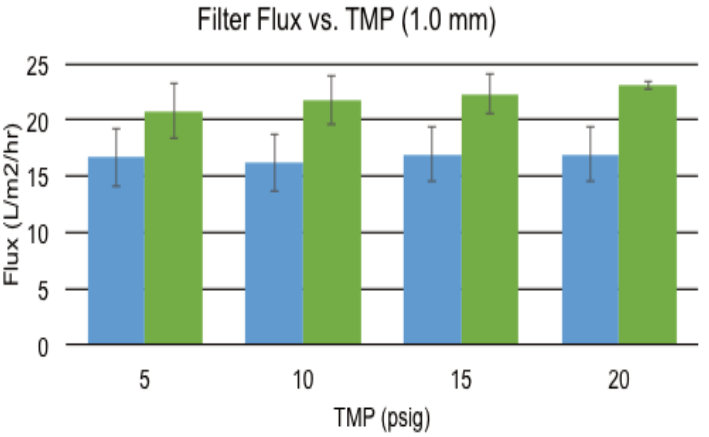

Figure 6 Results of increasing TMP via backpressure on 1.0 mm inner diameter HF membranes

Conclusions

Here, we have described the process of IgG concentration using HF filter modules containing fibers with inner diameters of 0.5 and 1.0 mm. This application note has shown that with SpectrumLabs.com HF membranes, IgG concentrations can be brought up to 350 mg/ml. In addition, we have shown the differences in performance between the 0.5 and 1.0 mm inner diameter membranes.

These experiments have shown that filter modules containing 1.0 mm inner diameter fibers are better-suited for the concentration of Abs. Use of the 1.0 mm inner diameter fibers result in both lower pressure drops and higher flux rates, allowing for shorter processing times.

We have also shown that applying backpressure to the process does not significantly improve filter flux. In some cases, backpressure can decrease filter flux. Of course, each individual process will require its own set of experiments to optimize the parameters, but these results provide a good starting point (i.e., 6000 – 8000 s^{-1}, no applied back pressure).

In conclusion, HF membranes from SpectrumLabs.com are able to provide high Ab concentrations in a quick, gentle, and robust manner, ultimately minimizing product loss. Additionally, product isolation should be maintained at high levels due to the geometry and drain ability of HF filter modules, further increasing product yields. Taking these experiments with IgG all together, similar processes can be applied to the purification of mAbs.

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