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Strategy for Optimization and Scale-Up

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Successful launch of several high-profile monoclonal antibody (MAb) products in recent years has increased attention to their potential—and to the challenges of producing them for therapeutic injection. Because MAb functions are very specific, they have emerged as important therapeutic molecules in the biopharmaceutical industry. Sale of MAbs is projected to reach US$16.7 billion by 2008 (**1**). It is often necessary to administer large amounts of a MAb drug to achieve sufficient therapeutic affect, but delivery is limited by subcutaneous injection volume. Therefore, producing a product with a high concentration of MAb in a small injection volume is key to success. Reliable, scalable processes must be developed to produce such high concentrations without compromising product quality. There have been reports of successful high-concentration formulations with albumin (**2**) and polypeptides such as human interleukin-1 receptor antagonist (**3**), but few studies about high MAb concentrations have been published or discussed in industry forums.

We describe here a well-defined experimental approach to optimizing and scaling up the ultrafiltration/diafiltration step in a MAb drug formulation in a clinical manufacturing process. We developed a robust, scalable formulation step that allowed us to produce clinical supplies at product concentrations up to 183 mg/mL of protein.

EXPERIMENTAL METHODS AND MATERIALS

To develop this process we used ABthrax protein, a MAb in clinical development for neutralization of anthrax toxin. The ABthrax UF/DF process comprised three steps: initial concentration to minimize the diafiltration volume, diafiltration into the appropriate formulation buffer, and final concentration to the desired formulation concentration. Therefore, we divided process optimization experiments into multiple segments. First, we performed total recycle evaluations to determine optimum transmembrane pressure (TMP) and feed flux in the initial buffer. We then carried out volume reduction experiments to determine optimum diafiltration concentration and minimize process time. We repeated
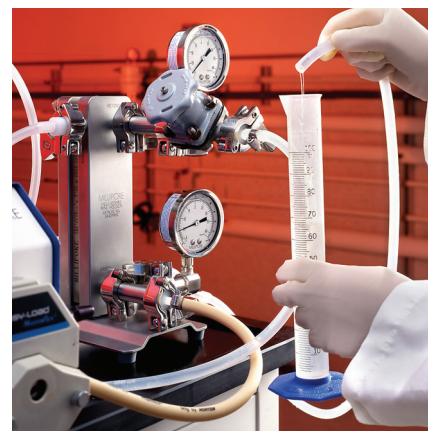
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the total recycle experiment for optimum TMP at the end of the diafiltration step to determine optimum conditions. Finally, we evaluated product recovery using three different methods.

Our experimental set-up is illustrated in Figure 1. It included Millipore's Pellicon 2 tangential flow mini-cassette with Biomax polyethersulfone 50 kDa membrane with C screen. Compared with regenerated cellulose membranes, Biomax membranes can stand rigorous cleaning with stronger caustic solutions such as 0.5 N NaOH. Typically, a molecular weight cut-off (MWCO) of 3–5 times tighter than the product size (for example, 30–50 kDa) cutoff is chosen for a MAb UF/DF application (**4**). We used a pore size of 50 kDa to retain the antibody while allowing high flux. Instead of "A screen," we chose "C screen" to handle the protein solution

PRODUCT FOCUS: MONOCLONAL ANTIBODIES, PROTEINS

PROCESS FOCUS: DOWNSTREAM PROCESSING

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KEYWORDS: ULTRAFILTRATION (UF), DIAFILTRATION (DF), HIGH-CONCENTRATION FORMULATIONS, OPTIMIZATION

LEVEL: INTERMEDIATE
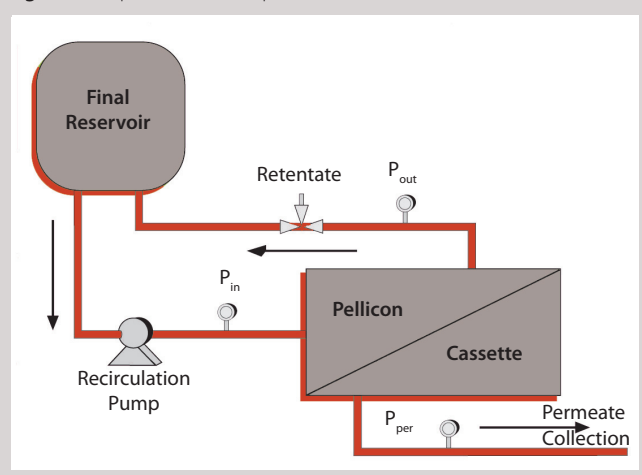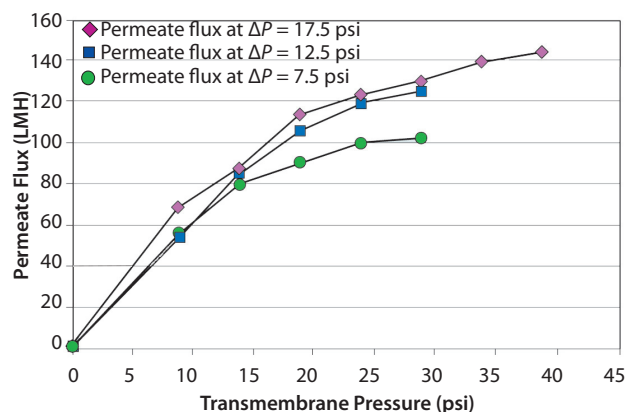
Figure 1: Experimental set-up

Figure 2: Permeate flux compared with TMP at various ΔP values (flux vs. TMP at $1 \times VCF$; Biomax-50 mini, C screen)

because we expected higher viscosity resulting from the higher protein concentration. We chose the mini-cassette because of its scalability (**5**) and to minimize feed volume necessary for the experiments.

RESULTS AND DISCUSSION

We carried out TMP and feed flux optimization in total recycle mode. We chose the highest feed flux, based on prior experience, and operated the system at the lowest TMP. After the system stabilized, we collected permeate flux and permeate samples for the A_{280} assay. We then increased the TMP to the next set point and repeated the process. Upon completion of the TMP excursion study at the highest feed flux, we decreased the feed flux and repeated the TMP excursion study. The initial feed volume was 4.1 L at a protein concentration of about 6 mg/mL, which would be a reasonable loading of approximate 250 g/m². Based on the results of permeate flux versus TMP experiments as shown in Figure 2, we determined from the knee point of the curve that the optimum TMP for the initial concentration was about 19–22 psi. The pressure drop, or ΔP, from the cassette feed to retentate was 15–17 psi.

Figure 2 shows the effect of ΔP on permeate flux. Increasing ΔP from 7.5 to 12.5 psi improved the permeate flux by 17%. However, increasing ΔP from 12.5 to 17.5 psi did not result in a corresponding percentage increase in permeate flux. Based on those results, we selected operating conditions for the initial concentration: 19 psi TMP, 400 L/m²/h (LMH) cross flow with

a corresponding ΔP of 12.5 psi. The effect of TMP on ABthrax retention was predictable. At higher TMP (in a pressure-independent regime with high polarization), protein retention is lower than that at lower TMP. The protein retention was 99.94% at the selected operating conditions: TMP at 19 psi, cross flow at 400 LMH, ΔP at 12.5 psi.

We carried out concentration and diafiltration experiments at a TMP of around 20 psi and a ΔP of 12–15 psi. The permeate flux dropped as the concentration of protein increased in both the starting buffer and the final buffer. A drop in permeate flux during protein concentration is potentially due to an increase in the wall concentration and the resultant increase in osmotic pressure from the protein polarization layer (**6**).

We concentrated the protein at these conditions and plotted permeability against volumetric concentration factor (*VCF*), as shown in Figure 3. We collected *VCF* versus permeate flux data from two experiments. One was carried out in the initial buffer, and another was carried out in the final buffer. We determined that the optimum volumetric concentration factor for starting the diafiltration process (VCF_{df}) was 5.3-fold in the initial buffer. We calculated the VCF_{df} by plotting the protein permeate flux versus volumetric concentration factor, determining the gel point VCF_g by extrapolation where the permeate flux is theoretically 0, and dividing the VCF_g by e (2.718). Thus, $VCF_{df} = VCF_g/e$ to give the optimum volumetric concentration factor (**6, 7**).

To get the VCF_{df} information in the final buffer, we diafiltered the protein by constant volume diafiltration using five volumes (five times the retentate volume) of diafiltration buffer. We diluted the retentate using the diafiltration buffer and repeated the permeate flux versus *VCF* experiment. VCF_{df} in the final buffer was 7.6-fold. We selected the lower of the two values of VCF_{df}'s in the initial and final buffers to simplify the UF/DF operation.

Selecting the best possible location for the diafiltration step is critical to minimizing the processing time for the UF/DF step. If the protein concentration at the diafiltration step is chosen as less than the C_{df} determined by C_g/e, the resultant process requires more buffer and takes longer to process. On the other hand, if the protein concentration at the diafiltration step is chosen as greater than the optimal C_{df}, the process flux is very low and takes longer to process.

Figure 4 illustrates the effect of TMP on permeate flux after 5.3-fold concentration in the starting buffer and the final buffer (following diafiltration with five diavolumes). We increased permeate flux as TMP increased. After diafiltration at a given TMP, permeate flux was lower than that in the starting buffer. The optimum TMP was about 20 for the starting buffer and about 25 psi for the final buffer. We chose an operating TMP at 20 psi to avoid potential polarization and to avoid high system pressure when scaling up.

During the final concentration to about 183 g/L the TMP increased to

Figure 3: VCF_{df} determination in the initial and final buffers (permeability vs. volumetric concentration factor, ΔP = 12.5–17 psi; TMP = 19–28 psi; Biomax-50 mini, C screen)

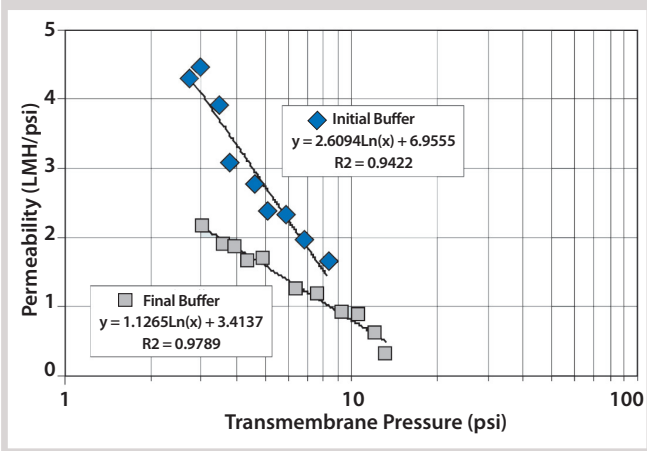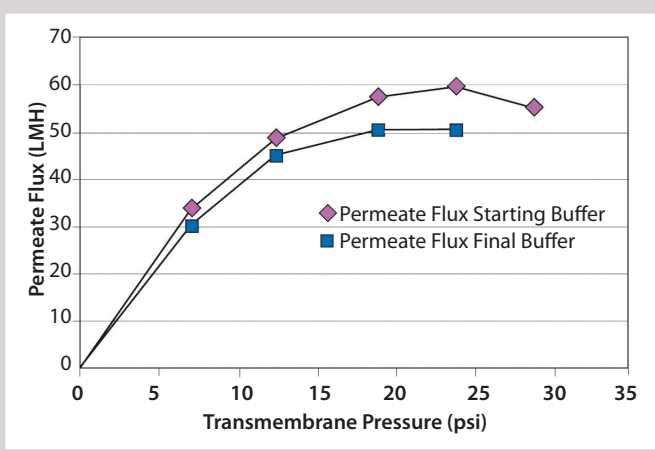**Figure 4:** Permeate flux compared with TMP in the initial and final buffers (permeate flux vs. TMP at 5.3 × VCF, ΔP = 14 psi, Biomax-50, C screen)

26 psi and the ΔP increased from 14 to 43 psi. The average permeate flux during the trial was 84 L/m²/h. At the conclusion of the final concentration step, we closed the permeate valve and allowed the system to recirculate for about 20 minutes to depolarize the membrane. We then pumped the retentate out of the system into the product recovery container. We added formulation buffer to the feed vessel just as the retentate was pumped out to prevent air from being pumped into the system, which may result in foaming and protein denaturation. We then collected the buffer in aliquots and assayed. At the conclusion of 30 mL of plug flow chase, we added about 100 mL of buffer to the feed vessel and recirculated with the permeate valve closed for approximately 20 minutes.

We then drained the system and assayed samples. The product yield in our retentate was 80% at 183 mg/mL and 92% at 167 mg/mL with one 30 mL plug flow buffer chase included. Recovery was approximately 100% at 106.5 mg/mL with the 30 mL plug flow buffer chase and 100 mL buffer recirculation included. Although more product may be recovered using a buffer recirculation rinse, the drawback of that approach is that product concentration in the flush tends to be at a significantly lower protein concentration. That results in a dilution of the product pool when it is added back.

Protein at very high concentrations tends to adhere to pipette tips because it is very viscous. A small amount of protein adhered to a pipette tip can affect assay results significantly. For that reason, we recommend against performing a 100× or 200× dilution on the high protein concentration sample for running the assays. We recommend a large volume 2× dilution first with subsequent dilutions of 10× each to minimize assay errors. Also, if the density of the product at these high concentrations is known, the assay can be performed by measuring the weight of protein sample added to the buffer for the assay measurement.

Our total processing time was two hours at a loading of 250 g/m², a very reasonable process time for a UF/DF step at scale. Further, we observed no detrimental effects on the protein at this high concentration based on analytical results. We conducted an ABthrax in-process stability study on the UF/DF pool at a protein concentration of 126 mg/mL and found, using specific assays to assess the biochemical stability of the product, that the antibody in the pool was stable for at least seven days at 4°C and at room temperature.

SUCCESSFUL OPTIMIZATION

Producing highly concentrated MAbs can be challenging, but it is essential for therapies intended for subcutaneous administration because doses are limited by injection volume. To reduce process time and maintain high product yield, successfully optimizing the ultrafiltration/ diafiltration step to manufacture therapeutics at very high concentrations is critical. It can result in a more streamlined, cost-effective process.

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*Corresponding author **Robert Luo** is a senior scientist II in the purification sciences department at Human Genome Sciences Inc., 14200 Shady Grove Road, Rockville, MD 20850, 1-240-314-4400, x1489, robert_luo@hgsi.com; **Ruta Waghmare** is group leader and process-development scientist, and **Mani Krishnan** is program manager, virus and biomolecular clearance at Millipore Corporation; **Christopher Adams** is a bioprocess associate II, **Ernest Poon** is a bioprocess engineer II, and **David Kahn** is a director, in the purification sciences department at Human Genome Sciences Inc.*

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