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Application Note

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Reliable Concentration of Novel Biologics with Vivaflow® SU to Support Formulation Development

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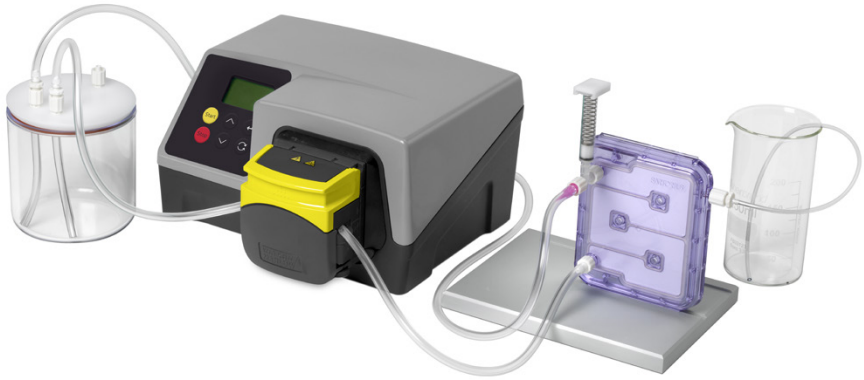
Abstract

Biological therapies are designed to target specific molecules or pathways in the body for precise and effective treatment of diseases such as cancer. When developing novel biologics, formulation development is a critical part of the process, to ensure safety, stability, and commercial viability. During formulation development, ultrafiltration is an invaluable technique to increase the concentration of dilute biologics from bulk material. In this study, Vivaflow® SU tangential flow filtration (TFF) cassettes were tested in a formulation development workflow and evaluated for concentration performance.



Introduction

For formulation development, novel biologics are often prepared as bulk material at low concentrations. Therefore, it is usually necessary to concentrate this material to the target concentrations required for screening in the test formulations[1].

To minimize the effort and cost associated with the development of biologics, achieving a high protein recovery rate at each step of the process is a top priority[1,2]. This includes the concentration of bulk material, which should be optimized to suit the unique characteristics of the candidate protein. Choosing the optimum ultrafiltration membrane chemistry and molecular weight cut-off (MWCO) is critical to ensure maximum protein recovery.

One of our novel biologics, a 59 kDa recombinant protein, required a concentration step prior to formulation development for Phase I clinical trials. We concentrated this protein by TFF using Vivaflow® SU and characterized the retentate samples by SDS-PAGE and HPLC to determine which membrane chemistry to use for bulk processing.

Methods

Tangential Flow Filtration (TFF)
Bulk samples of the candidate protein were concentrated by TFF using Vivaflow® SU cassettes with 10 kDa MWCO Hydrosart® regenerated cellulose (RC) or 10 kDa MWCO polyethersulfone (PES) membranes. To assess TFF performance, 90 – 100 mL samples were concentrated at 2 bar (retentate) at room temperature, until a volumetric concentration factor ≥4X was reached. For bulk processing, two separate batches of the candidate protein were concentrated at 2 bar (retentate), with the feed reservoir kept at room temperature (Batch 1) or incubated on ice (Batch 2) until a volumetric concentration factor of ~6.6X was reached.

SDS-PAGE
Samples collected before and after TFF were diluted in LDS sample buffer (NuPAGE, Thermo Fisher Scientific) and incubated at 70 °C for 3 minutes. Proteins were resolved on a 4-12% NuPAGE Bis-Tris gel (MOPS SDS running buffer, 250 V, 50 min) alongside molecular weight standards (Precision Plus, Bio-Rad) and visualized by Coomassie staining (Invitrogen Colloidal Blue, Thermo Fisher Scientific).

HPLC
Chromatographic separations were performed on an Agilent 1260 HPLC instrument using a detection wavelength of 280 nm for analysis.

A Biozen 3 µm dSEC-2, 4.6 × 300 mm, 200 Å column was used for size exclusion chromatography (SEC). The mobile phase was 100 mM sodium phosphate, 200 mM sodium chloride, 5% 1-propanol, pH 7.4. Flow rate 0.6 mL/min; isocratic elution; run time 12 minutes. During separation, the column oven temperature was set at 25 °C and the sample temperature was 5 °C.

For ion exchange chromatography (IEX), a MabPac SCX-10, 4 × 150 mm, 5 µm column was used. Buffer A was 50 mM sodium acetate, pH 4.5 and Buffer B was CX-1 pH Gradient Buffer B, pH 10.2 (Thermo Fisher Scientific). Flow rate 0.6 mL/min; gradient elution (0 min 0% B, 1 min 0% B, 13 min 50% B, 14 min 50% B, 14.1 min 0% B); run time 18 minutes. During separation, the column oven temperature was set at 20 °C and the sample temperature was 5 °C.

For data processing, % peak values were calculated for each peak area relative to the sum of all peak areas. High molecular weight fraction (HMWF) and low molecular weight fraction (LMWF) were determined by the total area of all peaks eluting before and after the main peak, respectively. Acidic and basic protein variants were determined using the total area of all peaks eluting before and after the main peak, respectively.

Results

Process Optimization and Bulk Processing
To ensure minimal loss of our protein during concentration, we first evaluated the TFF performance for Vivaflow® SU with 10 kDa MWCO RC or PES membrane chemistries.

During a 20 minute process, the permeate flow rate was similar for both membrane materials. However, we only observed complete (100%) recovery of our protein when it was concentrated using the PES membrane (Table 1). Our candidate protein was not detected in the permeate for either membrane, suggesting that the lower recovery observed after TFF with RC may have been due to non-specific adsorption. We therefore chose PES to concentrate the remaining bulk material for formulation screening. During bulk processing, we found that complete protein recovery after TFF was repeatable.

Table 1: *Protein samples were concentrated using Vivaflow® SU with RC or PES membranes and recovery was determined by measuring the absorbance of feed, permeate (not shown) and retentate fractions at 280 nm.*

		Process Optimization		Bulk Processing
Membrane Chemistry		RC	PES	PES
Feed	Volume	100 mL	90 mL	311 mL
	Concentration	3.5 mg/mL	3.3 mg/mL	3.5 mg/mL
Retentate	Volume	25 mL	20 mL	47 mL
	Concentration	12.1 mg/mL	14.9 mg/mL	23.6 mg/mL
Process Time		20 min	22 min	125 min
Permeate Flow		3.8 mL/min	3.2 mL/min	2.1 mL/min
Protein Recovery		86%	100%	100%

Protein Characterization

To confirm that the stability of our candidate protein was not affected by the concentration process, we compared the molecular weight and composition (SDS-PAGE, SEC) and charge variants (IEX) in samples before (feed) and after (retentate) TFF.

Analysis by SDS-PAGE (Figure 1) showed a similar band pattern for both feed and retentate samples, with a dominant band migrating to the expected molecular weight. There was no evidence of protein degradation.

The candidate protein was detected as a major peak in all SEC elution profiles, confirming that the protein purity in the feed samples from Batches 1 and 2 was 84.2% and 88.0%, respectively (Figure 2A). The retentate samples showed a minimal (≤0.5%) loss of the main peak, accompanied by an increase in the high molecular weight fraction (%HMWF). This suggests that some protein aggregation occurred during TFF. In Batch 2, the relative increase in %HMWF was noticeably less than in Batch 1. This can be explained by the lower incubation temperature of the Batch 2 feed sample during TFF (feed reservoir incubated on ice), as the protein under investigation is known to be temperature sensitive. Furthermore, the lower retentate concentration for Batch 2 compared to Batch 1 (25.2 mg/mL vs. 31.5 mg/mL) may also have mitigated aggregation.

Figure 1: *Following bulk processing with Vivaflow® SU, the candidate protein (arrow) in feed and retentate samples was assessed by SDS-PAGE. M, molecular weight standards; 1, Batch 1; 2, Batch 2.*

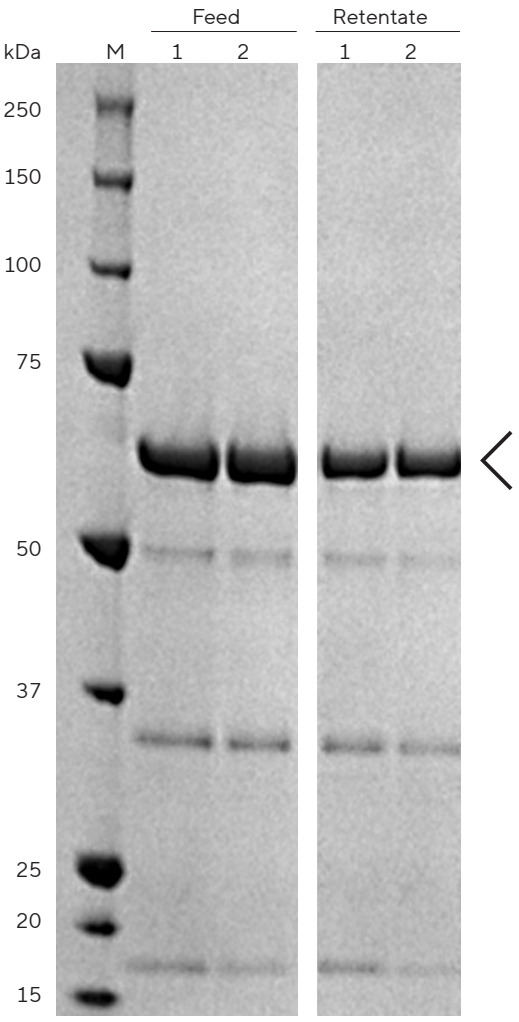
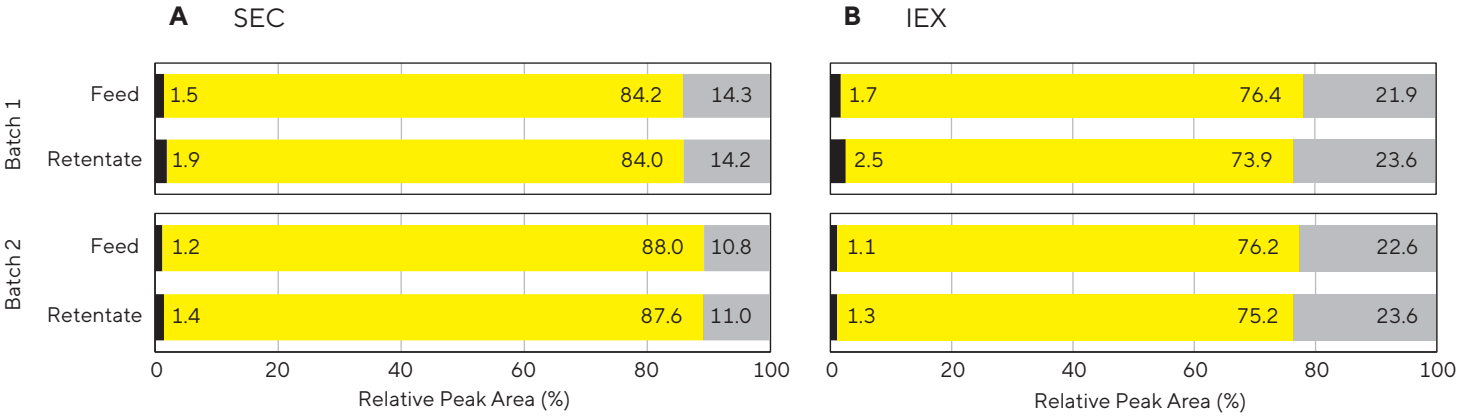


Figure 2: *Feed and retentate samples were assessed by SEC (A) to determine the relative compositions of the candidate protein (yellow), HMWF (black) and LMWF (grey), and by IEX (B) to determine the relative compositions of the candidate protein (yellow), acidic variants (black) and basic variants (grey).*



A similar pattern for the major peak corresponding to our protein was observed in the IEX elution profiles (Figure 2B). After TFF, Batch 2 showed less loss of the candidate protein (1.4% vs. 3.3%) and less variability in the charge variant profile, suggesting that the protein in this sample was more resistant to chemical modification during the concentration process.

Conclusion

After TFF with Vivaflow® SU, we achieved complete recovery of our candidate protein with good stability and no evidence of degradation. It is likely that additional process optimization would further prevent the formation of high molecular weight aggregates. For example, performing TFF in a cold room, rather than only passively cooling the feed reservoir, could further improve the stability of our temperature-sensitive protein. Furthermore, as high protein concentrations and longer process times may also favor aggregated states[1,3], use of the continuous diafiltration and the modular capabilities of Vivaflow® SU should also be explored as a means of improving molecular stability.

In summary, we have found Vivaflow® SU to be efficient and easy-to-use for small scale concentration of our candidate protein. These TFF cassettes are an ideal solution for formulation development workflows and could even be useful for the exchange of biologics into the selected test formulations by diafiltration.

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