Evaluating a mixing solution for optimal feed handling in bench-scale tangential flow filtration

Application Note

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Abstract

Two mixing devices, the 10 L ProConnex® MixOne Carboy and the 10 L Tulip tank with magnetic stirrer, were evaluated for homogeneity of feed material during an ultrafiltration/diafiltration (UF/DF) process. To assess their capabilities, two different proteins were processed using the KrosFlo® RS 20 Tangential Flow Filtration System. The CTech™ FlowVPX® in-line analytical system was implemented to measure protein concentration continuously throughout the process. It was shown that both mixing systems demonstrated comparable results and achieved sufficient homogeneity of the products and thus a consistent in-line concentration with high overall process efficiency. Additional aspects of the mixing devices were examined, such as power efficiency, shear, and material composition. This high-level analysis revealed advantages of the ProConnex® MixOne Carboy that make it the preferred system for the homogenization of shear-sensitive materials in downstream processing.

Introduction

In the biopharmaceutical industry, the efficiency of mixing processes plays a critical role in ensuring the quality and consistency of products. This is particularly true in tangential flow filtration (TFF) systems, where the uniform distribution of an active pharmaceutical ingredient (API) in the treated solution is essential for optimal performance. Mixing efficiency directly impacts several key process aspects, particularly the uniformity of concentration, the likelihood of fouling, and the overall yield and quality of the final product.[1] Efficient mixing ensures that all components are evenly distributed, which is crucial for achieving consistent results and maintaining the integrity of the filtration process. Inadequate mixing can lead to “hotspots,” where the concentration of certain components is higher than desired, potentially causing membrane fouling and reducing the efficiency of the filtration process.

Moreover, the optimization of mixing parameters, such as impeller design, speed, and shear rate, is essential for maximizing the efficiency of TFF processes. Studies have demonstrated that advanced mixing designs can significantly reduce processing time, increase productivity, and improve product quality.[2]

Evaluation of mixing efficiency in a process requires tools that provide fast, reliable measurements of critical process parameters, which reveal information on product homogeneity. This capability can be achieved by the implementation of in-line process analytical technology (PAT) instruments, such as the CTech FlowVPX System, to measure concentration directly in real time. Such instruments make it possible to assess process components without prolonged testing and complicated calculations.

Furthermore, the integration of advanced PAT instruments in TFF systems, such as the RS 20, has shown significant improvements in process performance.[3] When integrated into a TFF system, the FlowVPX System’s real-time concentration measurements enable proactive control and reduce the likelihood of quality deviations. This enhances the robustness of the entire downstream process, ensuring that the target concentrations are consistently achieved and maintained.

In this paper, we examine the performance of two mixing systems: a 10 L ProConnex MixOne Carboy system and the Repligen 10 L Tulip tank with a standard magnetic stirrer. Each

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mixing system was paired with the RS 20 TFF System, and concentration was monitored continuously using the FlowVPX System to assess mixing efficiency and overall process performance. By examining the performance and operational considerations of each system, we aim to provide insights on implementing a mixing system for downstream processing steps in biopharmaceutical manufacturing.

Materials and Methods

The first mixing device was the Repligen 10 L Tulip tank, the standard vessel included with the RS 20 System. It utilizes a cross-shape magnetic stirrer attached to a vertical shaft, which is mounted to the upper surface of the vessel. This 10 L vessel is the simplest design offered by Repligen; larger RS systems include Tulip tanks with a two-level mechanical stirrer configuration and dual feed outlets for optimized flow control.

The second mixing device tested was the 10 L ProConnex MixOne Carboy system. It features a rigid carboy container equipped with Metenova Truelev Mixing Technology, ensuring low-shear mixing and efficient resuspension. The system is designed with a bearing-free, fully levitating mix head intended for shear-sensitive products. The Truelev technology uses no moving parts in the motor and no parts or o-rings that experience wear, providing maintenance-free operation.

The Repligen RS 20 TFF System was used to establish real application conditions of the mixing systems in the study. The CTech FlowVPX in-line analytical system was used to measure product concentration in real time, a key parameter of TFF process effectiveness.

Two test substances were used in the study. The first was human transferrin glycoprotein (Repligen Sweden AB, Lund, Sweden) with a molecular weight of 76 kDa, available as an aqueous solution with initial concentration of 20 mg/mL. The second substance was Herceptin trastuzumab (F. Hoffmann-La Roche AG, Basel, Switzerland), a monoclonal antibody with molecular weight of 145.5 kDa, formulated as an aqueous solution in Tris acetate buffer with pH 7.0 and initial concentration of 8 mg/mL.

The RS 20 System performed an ultrafiltration (concentration mode) run for each test substance; no diafiltration step was included. The run was repeated using the standard Tulip tank and the MixOne System. TFF parameters for each run are shown in Table 1 and Table 2.

Finally, the Tulip mixing device was set to 75 rpm (the lower limit of the RS 20 system). Both runs with MixOne device were started at 90 rpm, and the speed was gradually reduced to 40 rpm (Figure 1).

In-line Measurement System

The FlowVPX System was configured with a 10 mm Flow Cell for all runs with the following measurement method parameters: continuous reading at 280 nm, extinction coefficient of 1.25 ml/(mg · cm) for transferrin and 1.47 ml/(mg · cm) for trastuzumab, and no scatter correction or buffer correction.

Product concentration is considered a key parameter for determining homogeneity, and thus mixing efficiency. Specifically, if the concentration curve over time remains smooth during the TFF process, it can be inferred that the feed material is well mixed. If the concentration curve shows high amounts of noise, it is likely due to a non-uniform

Table 1. TFF parameters: human transferrin glycoprotein

Mixing Device	Tulip Tank	MixOne
Initial Volume	7.2 L	8.2 L
Initial Concentration	24.5 mg/mL	20.0 mg/mL
Target Concentration	130 mg/mL	220 mg/mL
Feed Flow Rate	1.4 LPM	
Transmembrane Pressure	1 bar	
Flow Path	Proconnex® Flow Path, 1/4 in I.D. (part no. GA-KIT-TFF-000180)	
Filter	TangenX® SIUS® flat sheet cassette (part no. XP030L01L)	
Filter Surface Area	0.1 m^2	
Molecular Weight Cut-off	30 kDa	

Table 2. TFF parameters: trastuzumab (Herceptin)

Mixing Device	Tulip Tank	MixOne
Initial Volume	8.0 L	
Initial Concentration	9.4 mg/mL	9.0 mg/mL
Target Concentration	90 mg/mL	105 mg/mL
Feed Flow Rate	1.8–2.0 LPM	
Transmembrane Pressure	1 bar	
Flow Path	Proconnex® Flow Path, 1/4 in I.D. (part no. GA-KIT-TFF-000180)	
Filter	TangenX® SIUS® flat sheet cassette (part no. XP030L01L)	
Filter Surface Area	0.1 m^2	
Molecular Weight Cut-off	30 kDa	

distribution of material in the solution, indicating an inefficient mixing system.

The concentration data collected by the FlowVPX System are further supported by the R^2 value, or coefficient of determination. The FlowVPX System measures the absorbance of light through the sample at multiple pathlengths, called section data, which is used to calculate concentration according to the Beer-Lambert law. The R^2 value indicates the linearity of the section data: an R^2 value near 1.0 indicates a near-perfect linear relationship and agreement with the Beer-Lambert law.

In order to be considered a valid concentration measurement, the R^2 must be at least 0.999. An R^2 value lower than this threshold may indicate inconsistencies in the feed material and thus insufficient mixing in the reservoir.

Results and Discussion

The essential parameters of TFF runs for the present study were collected from Repligen RS 20 System: transmembrane pressure (TMP), permeate flux, and feed weight. In addition to these, the system provides other important run parameters, including feed, retentate, and permeate pressure values; flow rates based on flow meters and pump speed; and temperature and conductivity.

Evaluation of the essential TFF parameters primarily focused on stability: a well-mixed feed reservoir is expected to yield data that either remains constant or changes steadily, in either case free of sudden, unexpected jumps in magnitude. In all process runs, TMP remained stable at the set value of 1 bar throughout the run (Figure 2, Figure 3). The permeate flux behaved similarly for both mixing devices: the maximum flux during runs with transferrin was slightly below 70 LMH, which decreased to about 40 LMH at the end of the run (Figure 4). In the trials using trastuzumab, the permeate flux quickly reached its maximum at 80 LMH but decreased to 9 LMH with the Tulip tank and 12 LMH with the MixOne System (Figure 5).

The in-line concentration measured by the FlowVPX System was quite consistent for both mixing devices (Figure 6, Figure 7). There was little to no noise observed in the concentration readings, which provides substantial evidence of effective mixing, as discussed earlier.

For both tested mixing devices, the R^2 value never fell under the 0.999 threshold (Figure 8, Figure 9). The Tulip tank demonstrated a slightly lower R^2 profile overall, with slightly more variability in the early stages for both substances. The MixOne system yielded excellent R^2 values in the early stages, followed by a slight decrease directly correlating to the gradual decrease of the mixer's rotation speed. However, even with about a 56% decrease in speed, the mixing system

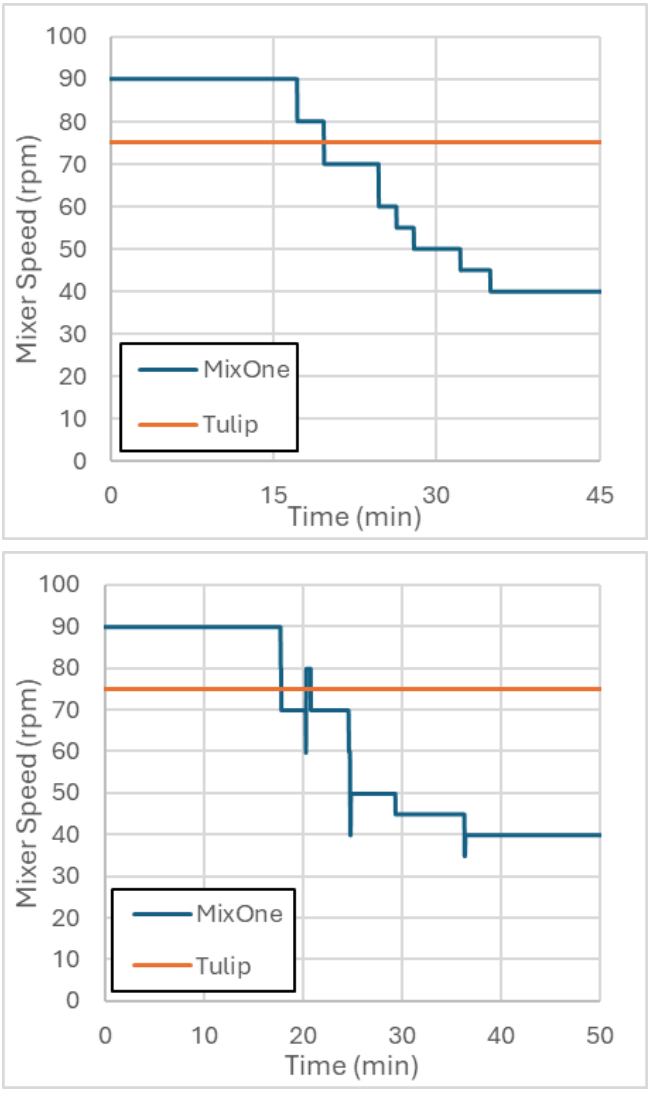

Figure 1. Mixing speed during transferrin run (top) and trastuzumab run (bottom).

still maintained sufficient homogeneity in the feed vessel. The behavior of the R^2 value may be a subject of future studies.

The feed tank weight was also recorded for the duration of each TFF run (Figure 10, Figure 11). Some of these curves exhibit sudden spikes or dips in the feed weight. These deviations from the smooth curve are not found in the in-line concentration graphs. Rather, in these cases, the weight measurement was likely influenced by environmental phenomena instead of properties of the process material. Such behavior exemplifies the risk of using feed weight to monitor an ultrafiltration process, as the equipment is sensitive to interference from other factors. In contrast, in-line concentration proved to be a reliable metric to control the process.

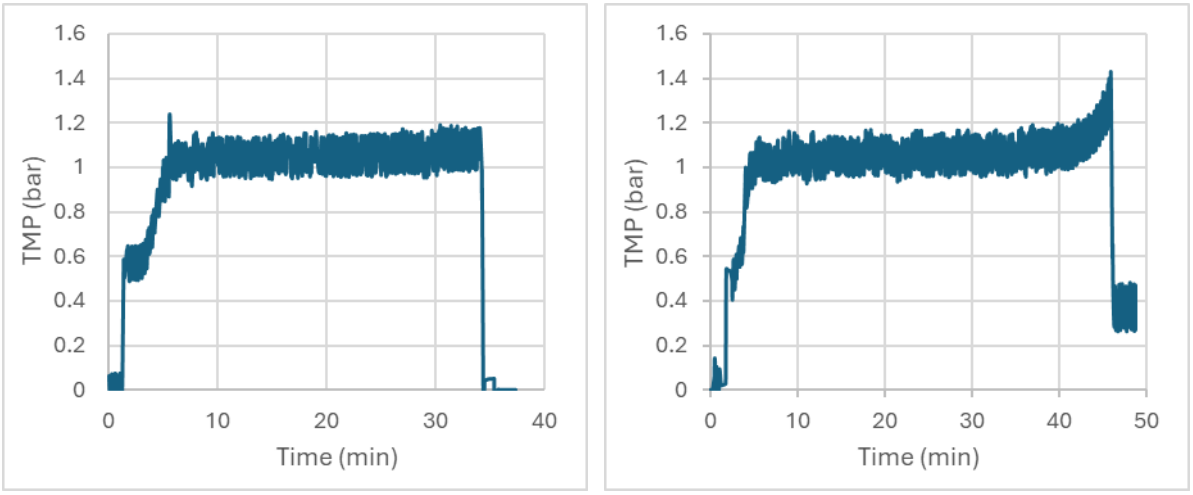

Figure 2. TMP during transferrin UF run using Tulip tank (left) and MixOne System (right).

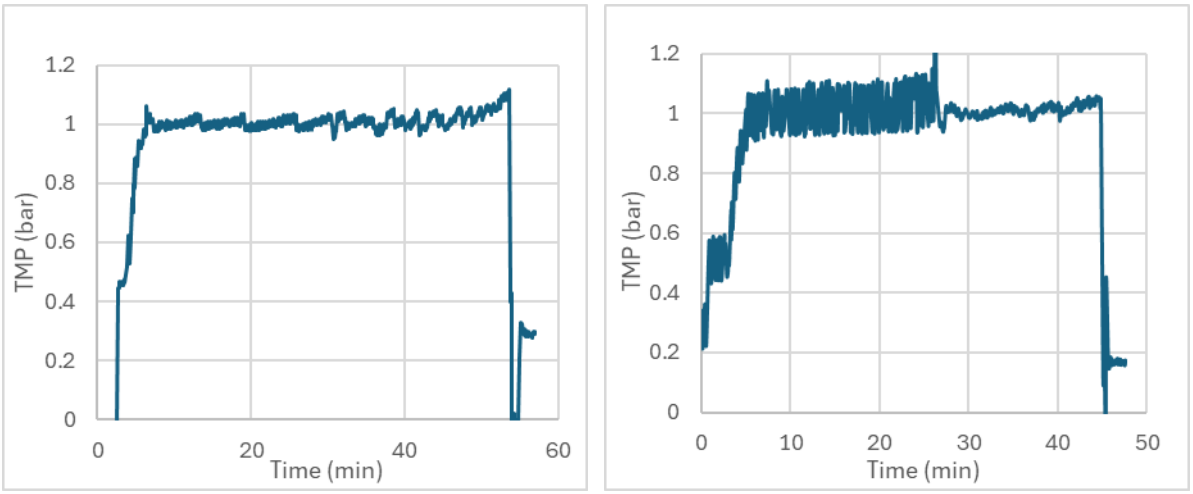

Figure 3. TMP during trastuzumab UF run using Tulip tank (left) and MixOne System (right).

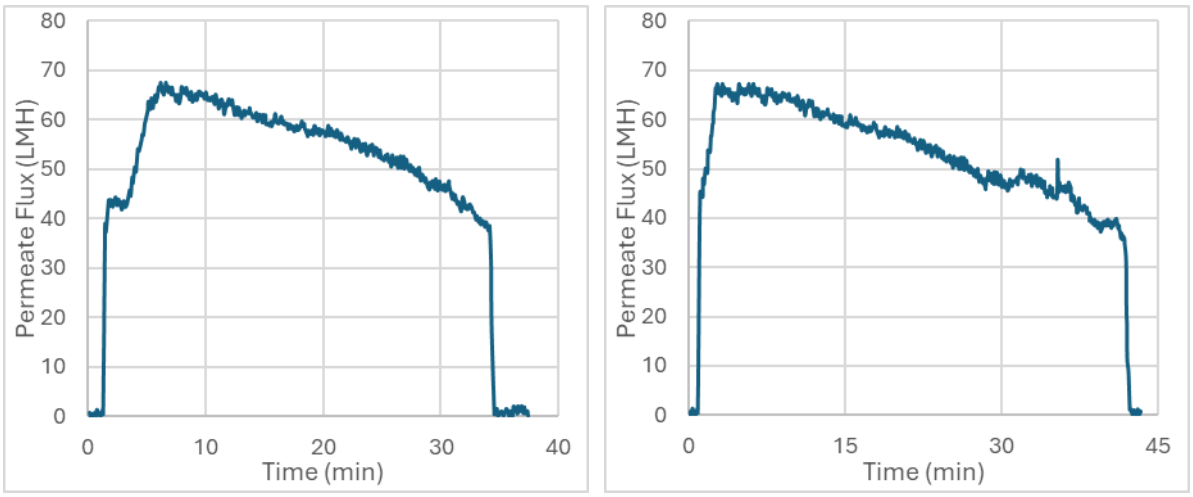

Figure 4. Permeate flux during transferrin UF run using Tulip tank (left) and MixOne System (right).

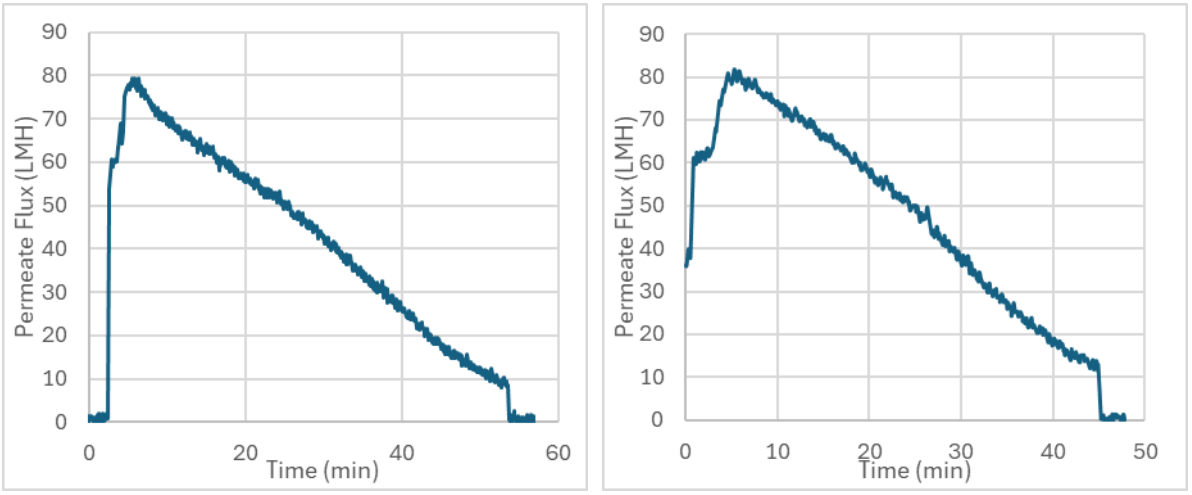

Figure 5. Permeate flux during trastuzumab UF run using Tulip tank (left) and MixOne System (right).

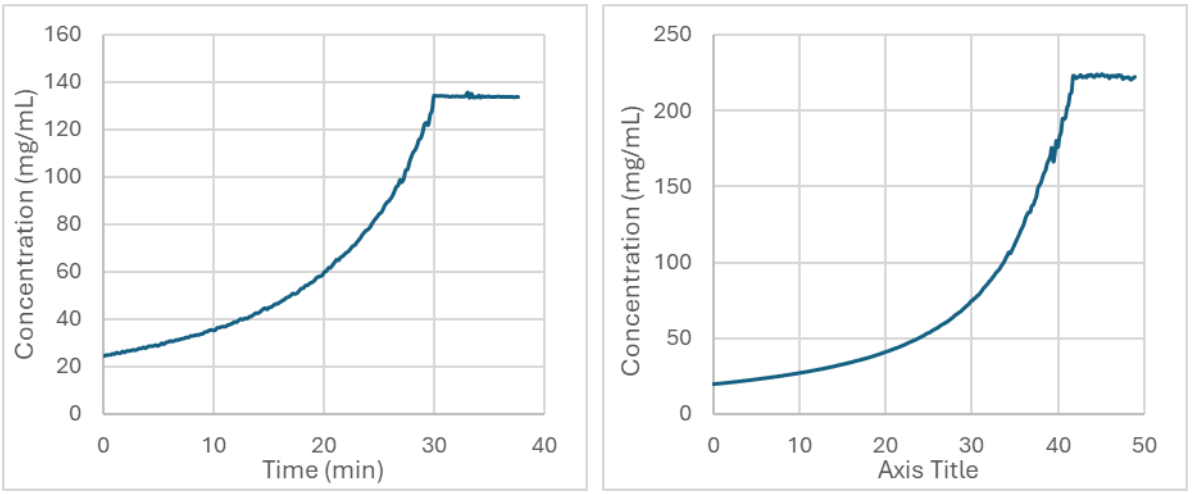

Figure 6. In-line concentration during transferrin UF run using Tulip tank (left) and MixOne System (right).

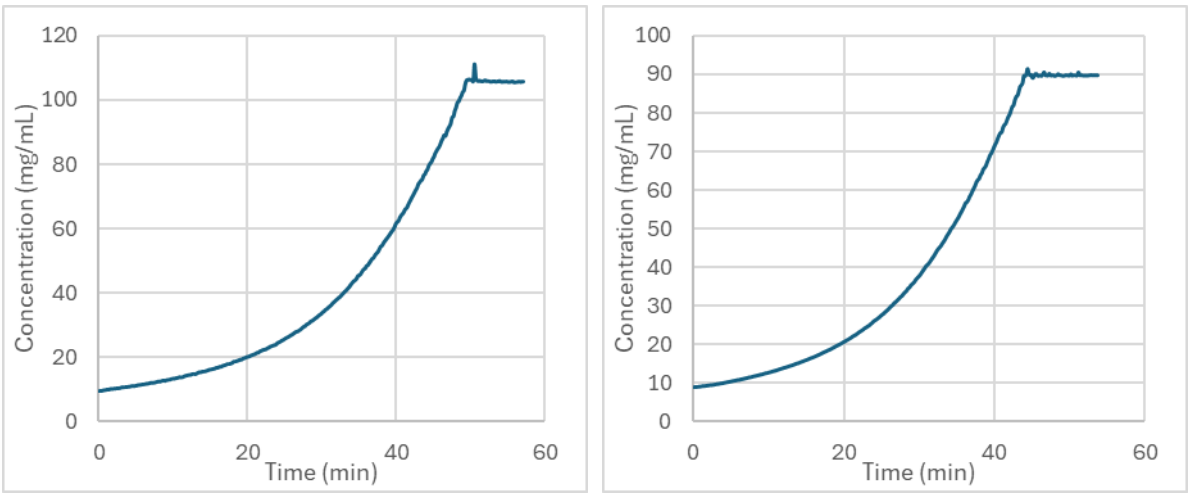

Figure 7. In-line concentration during trastuzumab UF run using Tulip tank (left) and MixOne System (right).

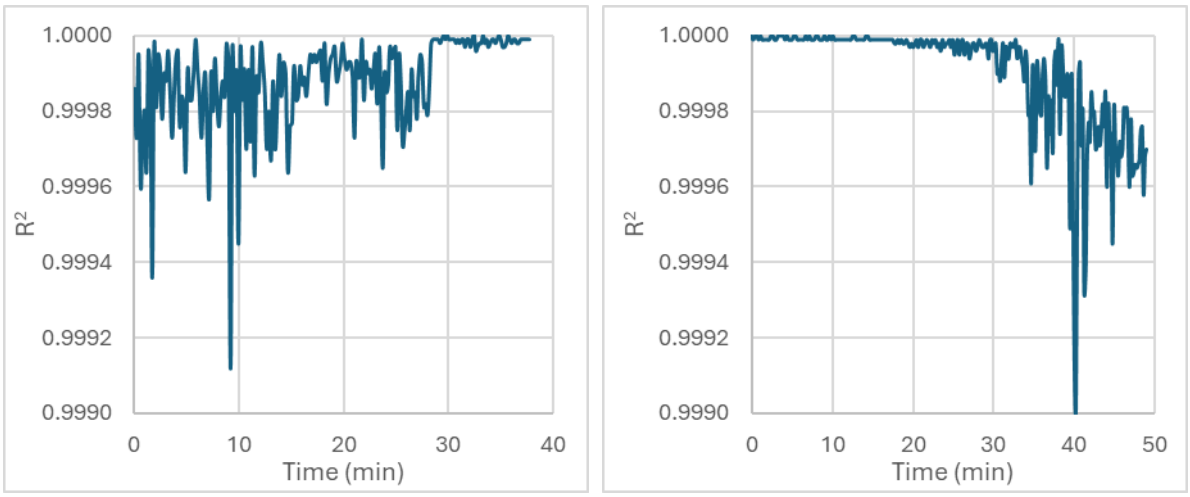

Figure 8. VPT R^2 value during transferrin UF run using Tulip tank (left) and MixOne System (right).

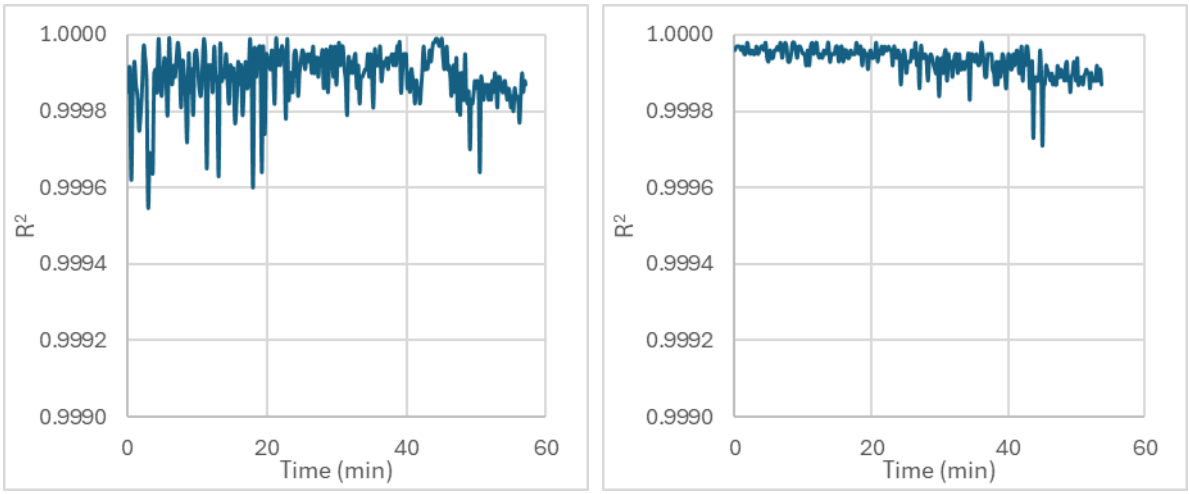

Figure 9. VPT R^2 value during trastuzumab UF run using Tulip tank (left) and MixOne System (right).

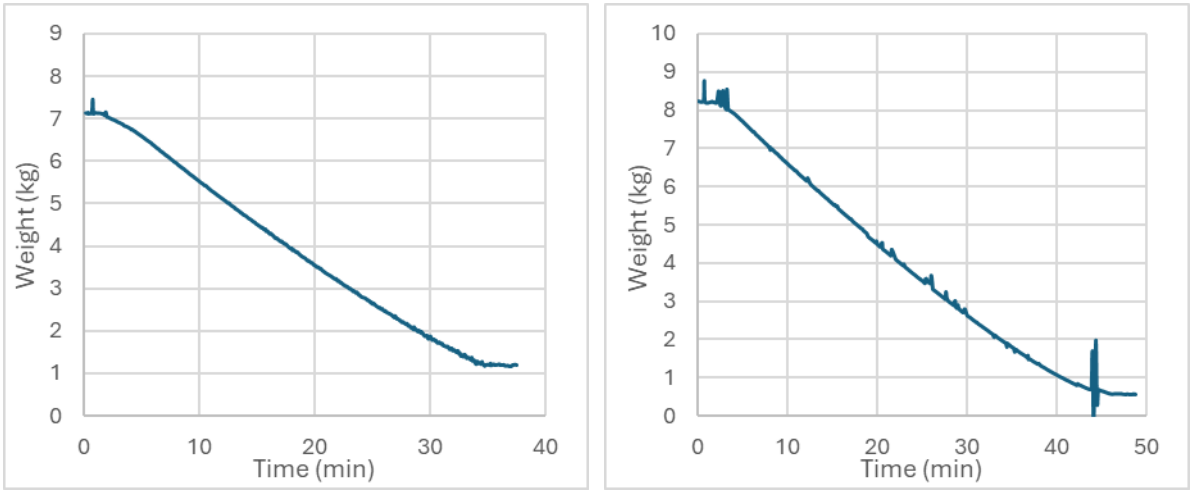

Figure 10. Feed tank weight during transferrin UF run using Tulip tank (left) and MixOne System (right).

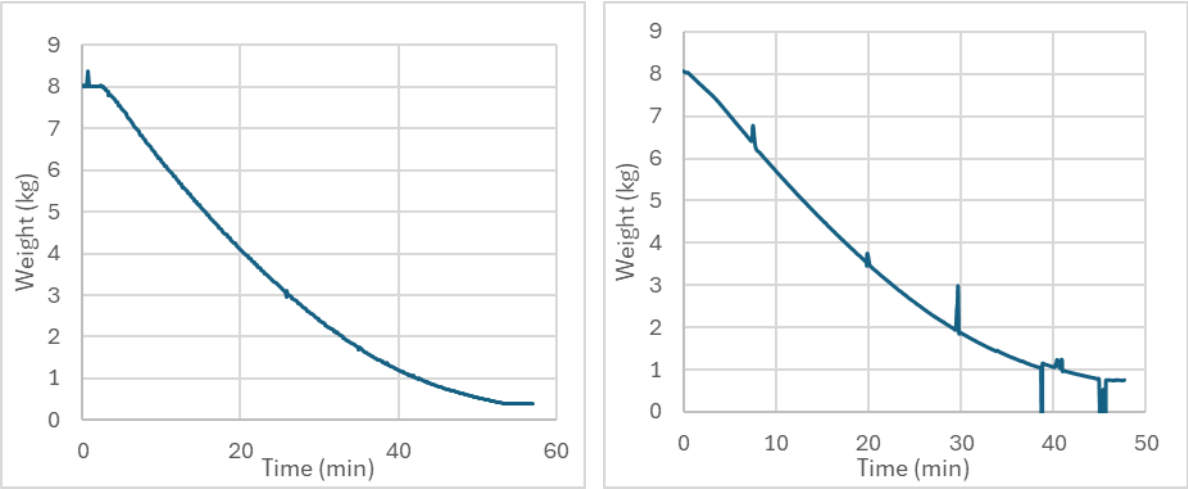

Figure 11. Feed tank weight during trastuzumab UF run using Tulip tank (left) and MixOne System (right).

Operational Considerations

In addition to the analysis of the TFF process runs above, it is important to consider several practical aspects of implementing a mixing system in downstream bioprocessing operations.

This study demonstrated that reducing the MixOne rotation speed by more than half (from 90 to 40 rpm) maintained sufficient mixing efficiency to keep the R^2 value within the recommended range for valid concentration measurements. As a result, power input can be reduced, thereby lowering operational costs and further minimizing potential mechanical stress on the API and matrix.

Previous studies have shown that the MixOne System delivers consistent low-shear mixing, which enhances its suitability for handling sensitive molecules.[4]

Slide bearing seals, commonly used in conventional vessel mixers, are designed to retain lubricants and prevent contaminants from entering the bearings. However, when made from plastic materials, these seals can degrade or become damaged, contributing to microplastic pollution.

The presence of microplastics in API solutions must be strictly avoided. The MixOne System features a bearing-less design, free of components that are prone to wear and tear in conventional mixing systems.

A bottom-mounted mixer like the MixOne carboy potentially allows for a lower minimum working volume compared to top -mounted systems (Figure 12), offering greater flexibility for process optimization and efficient use of equipment.

Figure 12. The bottom-mounted mix head in the MixOne System (left) allows for a lower minimum working volume than the top-mounted impeller in the Tulip tank (right).

Conclusion

It was shown that the MixOne System and the standard Tulip tank have comparable homogenizing efficiency under the experimental conditions tested in this study, as verified by the in-line concentration measurement of the FlowVPX system.

In practical considerations, the MixOne System offers several advantages compared to conventional mixers, including:

- power efficiency, with no observable decrease in performance at lower mixing speeds;
- low shear for sensitive molecules;
- a bearing-less design, free of components that may experience wear; and
- a bottom-mounted impeller, allowing for a lower minimum working volume than conventional mixers.

The study also provides support for the implementation of the FlowVPX in-line analytical system as a powerful tool for process development, not only for monitoring critical quality attributes of the process, but also when evaluating equipment for application suitability, operational efficiency, and safety.

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