Reducing Error Propagation in TFF Processes using the KrosFlo® KR2i RPM™ System

White Paper

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Introduction

Traditional tangential flow filtration (TFF) processes run based on mass calculations, which are prone to error due to instrument limitations and operator dependencies. The KrosFlo® KR2i RPM™ System uses the CTech™ FlowVPX® in-line spectrophotometer to monitor concentration in real time, reducing the risks associated with traditional TFF methods. This paper examines common practical errors that occur during a typical ultrafiltration/diafiltration (UF/DF) process and illustrates the advantages of using in-line concentration measurement as the process control method, compared to conventional mass balance calculations.

Background

Tangential flow filtration (TFF), or crossflow filtration, is one of the most widely used material-processing techniques in biotechnology, which separates components of a liquid solution or suspension using membranes. Unlike normal filtration, the liquid flow in TFF is parallel to the membrane surface, which minimizes retentate buildup (the “cake layer”) that can lead to membrane fouling. Molecules smaller than the pore size are forced through as a result of a pressure difference; larger molecules are recirculated into the feed vessel [1]. The common TFF applications in bioprocessing are ultrafiltration (UF), for increasing the product concentration, and diafiltration (DF), for buffer exchange or desalting. TFF is acknowledged as an essential part of most downstream processing (DSP) methods, thus requiring careful consideration from the beginning of process development and when scaling up the operation [2].

Traditionally, the UF/DF process is controlled based on mass calculations. Although universally adopted, this approach has considerable drawbacks, including the potential for complex computation, error susceptibility, operator dependence, and the need for off-line concentration measurement. These drawbacks would be significantly mitigated if the UF/DF process were completely automated and effectively controlled by the critical process parameters. Process automation provides risk reduction, strict process control, and the removal of the “human factor,” among other benefits [3]. The development of process automation is complicated, challenging, and demands large investments of time and effort from the end user.

The KrosFlo® KR2i TFF System is a widely distributed benchtop system for lab-scale TFF, including R&D and PD tasks in UF, DF, and more. Building on this powerful system, Repligen has introduced the KrosFlo KR2i RPM System: the world's first TFF system with in-line concentration management. It not only monitors critical parameters in the TFF process, but also enables active control over them. Real-time insights dramatically reduce the cost, time, and risk involved in the process. These process analytical features are made possible by the integration of the FlowVPX in-line spectrophotometer.

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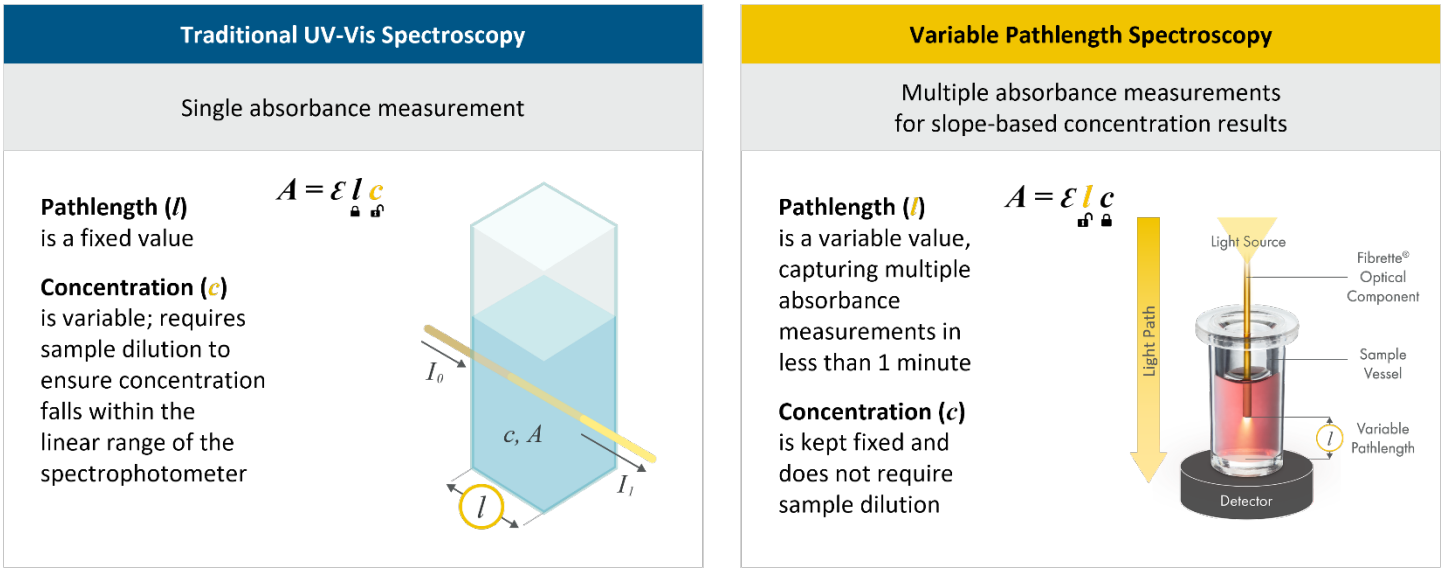
In-Line Analytics using Variable Pathlength Spectroscopy

The FlowVPX System uses Variable Pathlength Technology (VPT), an alternative approach to UV-Vis spectroscopy. Like traditional instruments, VPT is based on the Beer-Lambert law, where absorbance is proportional to both concentration and pathlength. However, unlike conventional UV-Vis spectroscopy where the pathlength remains constant, VPT varies the pathlength to adapt to the concentration of the solution, enabling the measurement of highly concentrated solutions without the need for dilution [4] (Figure 1).

Figure 1. Variable Pathlength Technology compared to conventional UV-Vis analytics



The FlowVPX System bases its measurements on the slope of absorbance versus pathlength rather than on absolute absorbance values, which allows for higher accuracy and eliminates the need for baseline correction in most cases.

By adding the FlowVPX instrument in the flow path of the KrosFlo KR2i TFF System (Figure 2), concentration can be constantly monitored and managed in real time using the dedicated KrosFlo® RPM™ Software (Figure 3). All system components are controlled through this software, which allows for full-fledged, walk-away automation of the KR2i functions based on the in-line concentration measurements of the FlowVPX System. In addition, the RPM software provides integrated data analysis with automatically generated graphs, charts, and tabular data collected from all inputs and outputs that are crucial for process understanding, development, and optimization. The software also allows the user to customize methods, export reports, and keep detailed trial logs [5].

Users who implement the FlowVPX System (as part of the RPM System or as a standalone instrument) should note that an essential prerequisite for accurate measurements is proper mixing of the feed solution. Neglecting to mix or mixing improperly usually leads to a lack of homogeneity in the feed, which drastically worsens the in-line absorbance measurements. When deploying the FlowVPX System in a process development lab, either a magnetic stirrer or a special laboratory scale stirring reactor should be applied to obtain the best results.

Materials and Methods

The KrosFlo KR2i RPM System was used to perform a TFF process. Product concentration was continuously monitored simultaneously via the conventional mass balance method, using precision scales, and in-line absorbance readings, using the FlowVPX System.

The TFF system was equipped with a SIUS PD 0.01 m^2 (EP) HyStream 30kD Cassette filter module (item # XP030LP1E). Masterflex L/S® Precision Pump Tubing L/S 14 was used to build the flow path. The FlowVPX 3 mm Stainless Steel Flow Cell was used with the FlowVPX System.

The TFF system was used in Concentration/Diafiltration/Concentration (C/D/C) mode using bovine serum albumin (BSA). The starting concentration was either 5 mg/ml (runs 1 and 3) or 10 mg/ml (runs 2 and 4); in all runs, the sample was concentrated to 200 mg/ml. Transmembrane pressure (TMP) was kept at 0.55 bar (7.98 psi) during runs 1, 3, and 4; TMP was 1.24 bar (17.98 psi) during run 2. The RPM software measurement method applied was Quick Mode at 280 nm wavelength with continuous read, using the standard BSA extinction coefficient of 0.67 $(mg/ml)^{-1}cm^{-1}$.

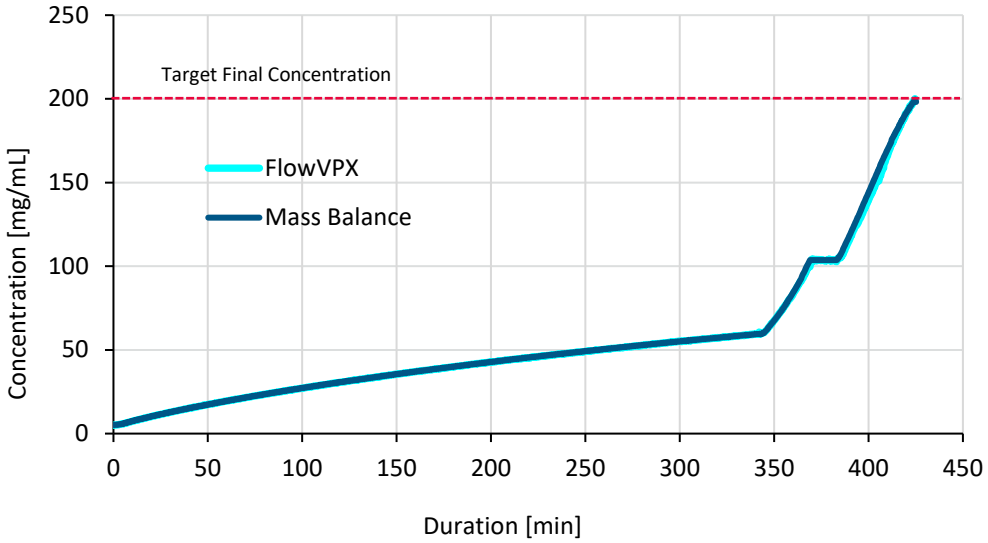
Figure 2. KrosFlo KR2i RPM Tangential Flow Filtration System

Figure 3. Example display of components inputs and outputs managed by RPM software

Results and Discussion

In Run 1, the first concentration target (C1) was 50 mg/ml; the sample was then washed with 0.3 diafiltration volumes (DV) before the final concentration step (C2). In this run, the VPT and mass balance methods produced very similar concentration data over the duration of the TFF process. The difference between the final concentration measurements by each method was less than 1% (Figure 4).

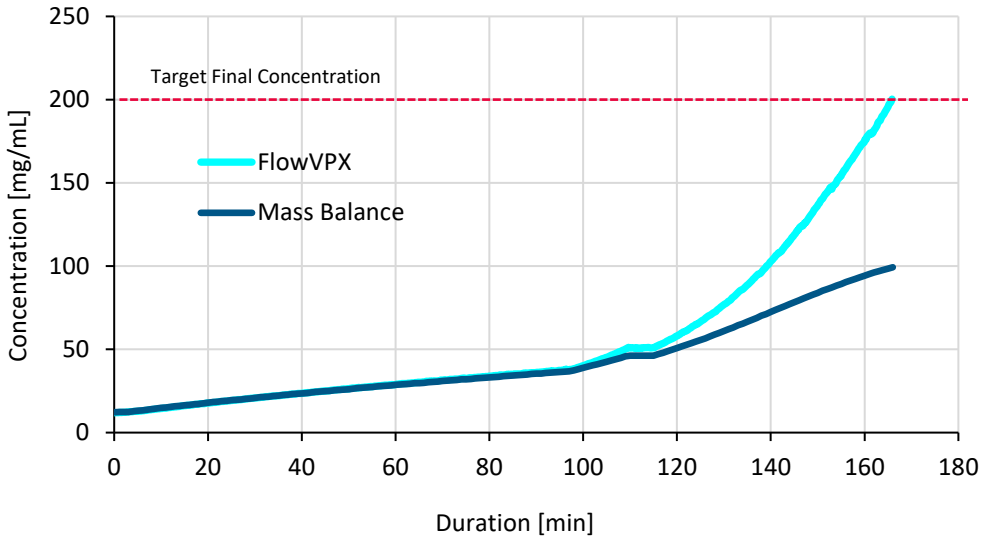
Figure 4. Run #1: In-line concentration measured by FlowVPX versus calculated concentration based on mass balance input



Run 1 demonstrates ideal parameters: no undesired interference took place, and the system was able to complete the process as planned. The following runs involve examples of common experimental errors due to inherent equipment limitations or human interaction with the equipment. These errors affect the performance.

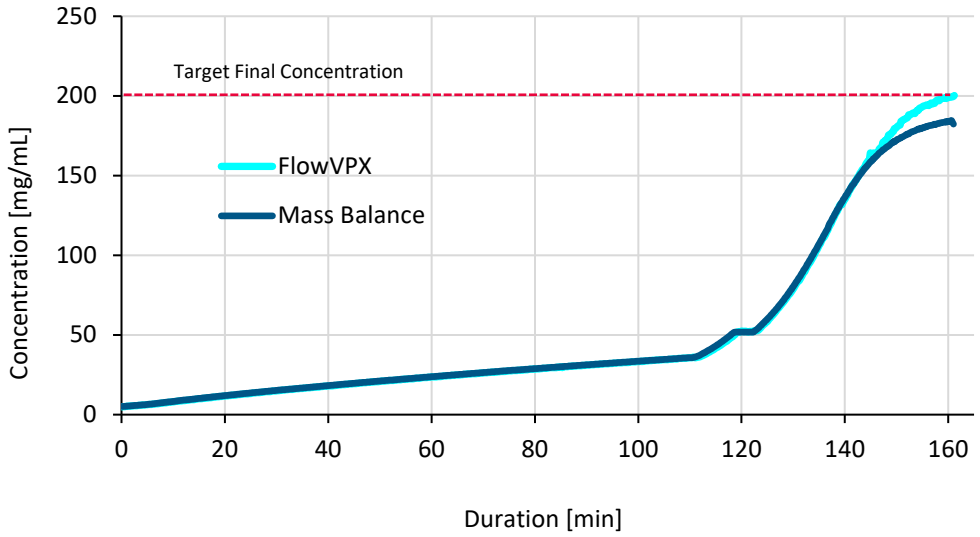
In Run 2, C1 was once again 50 mg/ml, followed by a 0.1 DV wash. However, approximately 100 g of liquid was not detected by the scale during the TFF process. This magnitude of error can occur if the user accidentally adds or removes any weight on the scale surface after the run begins; such a discrepancy can even arise if the system tubing is adjusted, affecting the force exerted on the vessel on top of the scale. In this case, the initial error caused a 10% difference between calculated concentration (mass balance) and measured concentration (FlowVPX) by the end of the diafiltration phase, which then grew to a 50% difference at the end of the process (Figure 5).

Figure 5. Run #2: In-line concentration measured by FlowVPX versus calculated concentration based on mass balance input



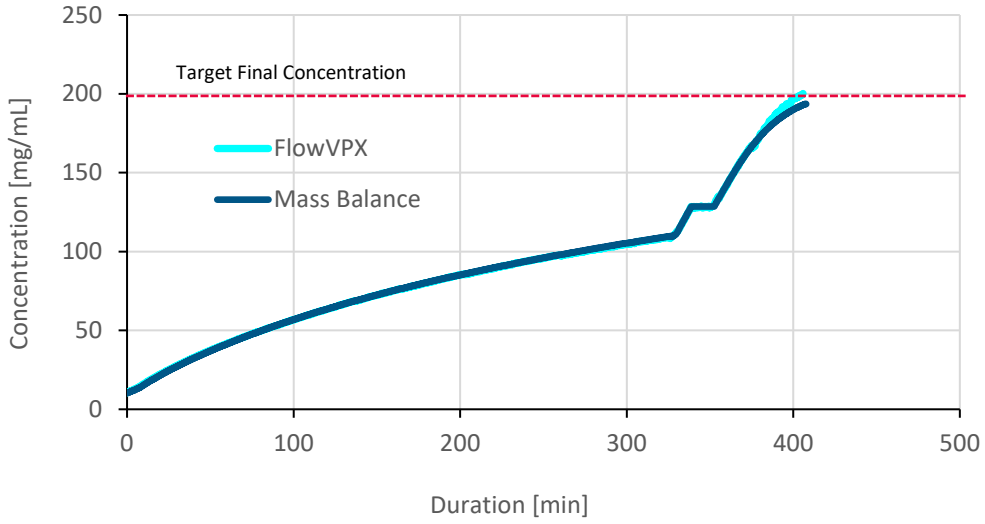
In Run 3, C1 was once again 50 mg/ml, followed by a 0.2 DV wash. Run 3 experienced a similar error to Run 2. The scales did not account for approximately 7 g of liquid during the TFF process, which resulted in a final difference of 9% between the VPT measurement (200.2 mg/ml) and the mass balance calculation (182.2 mg/ml) (Figure 6). This margin of error lies outside the acceptable range for most bioprocessing industry requirements [6].

Figure 6. Run #3: In-line concentration measured by FlowVPX versus calculated concentration based on mass balance input



In the final run presented here, C1 was 125 mg/ml, followed by a 0.2 DV wash. During the process, subtle, accidental changes caused a difference of 3.4% in the final product concentration (Figure 7). The practical errors that can produce an error of this magnitude may be undetectable; the analyst is unlikely to catch the mistake before it results in a significant deviation from the target value.

Figure 7. Run #4: In-line concentration measured by FlowVPX versus calculated concentration based on mass balance input



The margin of 3.4% between the measured and calculated concentration is not as severe as those in runs 2 and 3; it falls within the acceptable limits of many bioprocessing industry standards [6]. However, the nature of this error being virtually untraceable to its

cause emphasizes the risk inherent to the process when employing the traditional mass balance method to determine concentration.

Table 1. Summary of TFF process errors

Run #	Concentration (mg/ml)				% Off Target	
	Starting	Target Final	Final, Mass Balance	Final, In-line VPT	Mass Balance	In-line VPT
1	5	200	198.1	200.1	0.9%	0.1%
2	10	200	99.3	200.3	50.4%	0.2%
3	5	200	182.2	200.2	8.9%	0.1%
4	10	200	193.4	200.2	3.3%	0.1%

While these runs portray the results of various known and unknown errors in the TFF process, the common feature they all share is the consistency of the FlowVPX System. Because of its in-line design, the FlowVPX instrument is not susceptible to the same variability as the precision scales, and thus, it consistently achieved within ±0.2% of the target final concentration in all cases where the mass balance method yielded significantly greater errors.

Conclusion

The newly released KrosFlo KR2i RPM System provides an unparalleled level of accuracy, error mitigation, and ease of use. TFF that relies on mass balance calculations demands continuous attention from the operator and near-perfect handling of the system components; integrating the VPT instrument mitigates these issues with automated functionality that achieves greater process control. The system allows for a deep understanding of the TFF process at each step, providing numerous crucial advantages, including:

- Full-fledged walk-away automation based on in-line concentration control rather than on complicated mass balance calculations
- Considerable risk reduction due to constant in-line management and control
- Eliminating the need for time-consuming off-line measurements, which provides insights only after the process has already finished
- Full software integration, combining the innovative functions of the FlowVPX System with the versatility of the KrosFlo KR2i System
- High capacity for customization due to modular design and availability of ready-made components, such as flow paths, sensors, and containers (also available as single-use items)

These benefits make the KrosFlo KR2i RPM System an excellent choice for both existing and new FlowVPX and KR2i users looking to increase efficiency and reduce risk in their research and development TFF operations.

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