Application of In-Line Variable Pathlength CTech™ FlowVPX® System for Use of Concentration as a Feedback Loop

Brandon Goldberg
Repligen Corporation, Bridgewater, NJ, USA

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Abstract

Development of effective, commercially-viable process analytical technology tools requires the interconnection of processing equipment and analytical tools with robust methods and reliable controls. Regardless of whether the process is intended to be continuous, semi-continuous, or batch, automated process control and PAT can improve efficiency and product consistency in manufacturing. In this study, we demonstrate a system based on acquiring concentration via variable pathlength spectroscopy to control a tangential flow filtration (TFF) process. Real-time feedback of the concentration information from the variable pathlength spectrophotometer, the CTech™ FlowVPX® System, allows for adjustment of the feed-pump speed and the back-pressure valve. Samples were taken periodically during the process to compare to off-line instrumentation. Automated process control is demonstrated for various final concentration end-points.

Introduction to Variable Pathlength Technology (VPT)

Variable pathlength technology (VPT) is a UV-based technology and utilizes the Slope Spectroscopy® equation, which is derived from Beer-Lambert law ($A = \varepsilon lc$) and the concept of slope. The equation postulates that slope equals extinction coefficient multiplied by the concentration ($m = \varepsilon c$). The CTech FlowVPX System uses patented variable pathlength technology to search for 1 absorbance (1 Au) and measures 5 to 10 data points going down to create a slope. That slope divided by the extinction coefficient creates a concentration data point over time. This measurement happens continuously and approximately every 10 seconds so that concentration can be monitored in real time.

Beer-Lambert law:

$$A = \varepsilon lc$$

Slope Spectroscopy® equation:

$$m = \varepsilon c$$

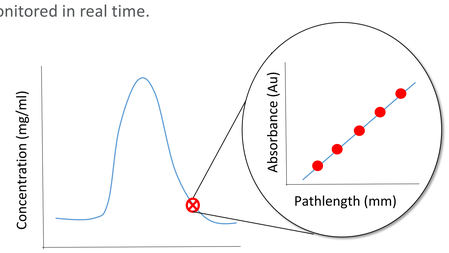

Figure 1. Beer-Lambert law and the Slope Spectroscopy equation.

The FlowVPX System can be used in-line as a process to provide concentration data in real time to be used as a feedback loop to control the process instead of using balances.

Figure 2. Proof of concept of concentration automation of TFF.

System Automation

The objective of this study was to create a fully automated TFF process solely based on concentration and compare it to the traditional TFF process which is based on the weight of the balances. To achieve this, Repligen's CTech FlowVPX System and KrosFlo® FS-15 System were used. Four TFF processes were performed: Two mass calculated processes and two TFF processes that were automated by concentration and using BSA as a sample. The first step was to ensure that each part of the TFF system was in communication with the FlowVPX System and its concentration readings. This communication was done through Java and JavaScript. A method then was developed to create parameters for each item to react. In this process, the main pump, the auxiliary pump 1, and the auxiliary pump 2 were being controlled through set concentration points.

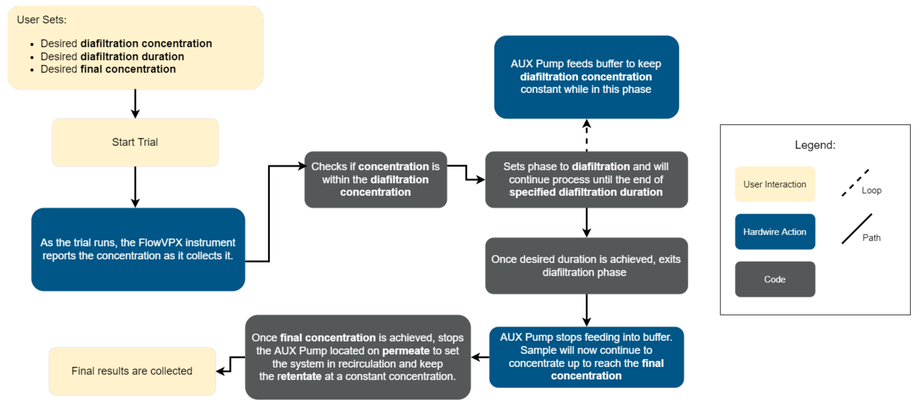

Figure 3. TFF automation workflow.

Data

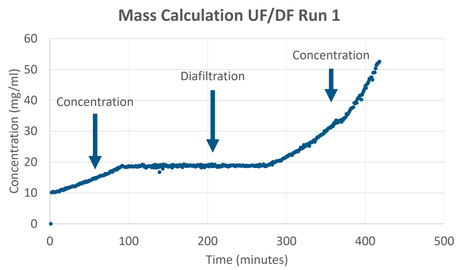

Figure 4. Mass calculation of UF/DF run 1.

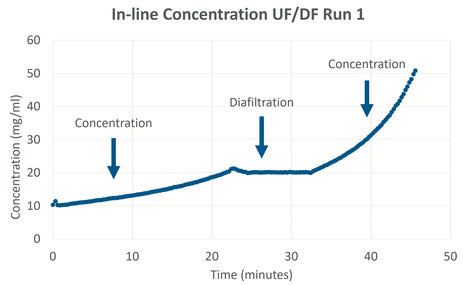

Figure 5. In-line concentration of UF/DF run 1.

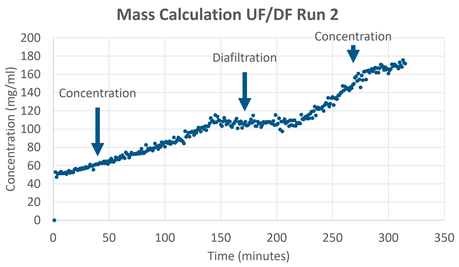

Figure 6. Mass calculation of UF/DF run 2.

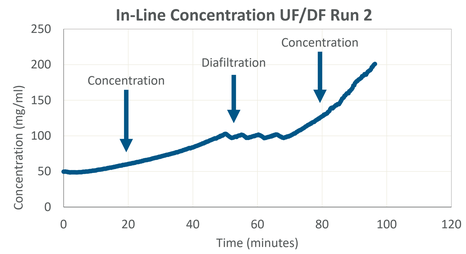

Figure 7. In-line concentration of UF/DF run 2.

- Two low concentration UF/DF runs at 10 mg/ml to 50 mg/ml using BSA
- Two high concentration UF/DF runs at 50 mg/ml to 200 mg/ml using BSA

Results

Mass calculation TFF for both low and high concentration resulted in a much larger percent difference than in-line concentration. In-line, concentration-controlled TFF automatically stopped diafiltration and ultrafiltration at each expected concentration while mass calculation TFF incorporated errors with the balances and holdup volume causing the TFF process to not hit the targeted concentration.

Table 1. Off-line data for low concentration run

Time Stamp	Expected Concentration	In-Line Concentration TFF	Mass Calculation TFF	In-Line Concentration Percent Difference	Mass Calculation % Difference
Start Ultrafiltration	10 mg/ml	10.30 mg/ml	10.14 mg/ml	N/A	N/A
Start Diafiltration	20 mg/ml	20.14 mg/ml	18.19 mg/ml	0.70%	9.05%
End Diafiltration	20 mg/ml	20.09 mg/ml	19.01 mg/ml	0.45%	4.95%
End Ultrafiltration	50 mg/ml	50.80 mg/ml	51.88 mg/ml	1.60%	3.76%

Table 2. Off-line data for high concentration run

Time Stamp	Expected Concentration	Inline Concentration TFF	Mass Calculation TFF	Inline Concentration Percent Difference	Mass Calculation % Difference
Start Ultrafiltration	50 mg/ml	49.67 mg/ml	50.93 mg/ml	N/A	N/A
Start Diafiltration	100 mg/ml	100.07 mg/ml	112.11 mg/ml	0.07%	12.11%
End Diafiltration	100 mg/ml	100.81 mg/ml	106.15 mg/ml	0.81%	6.15%
End Ultrafiltration	200 mg/ml	200.21 mg/ml	171.77 mg/ml	0.11%	14.12%

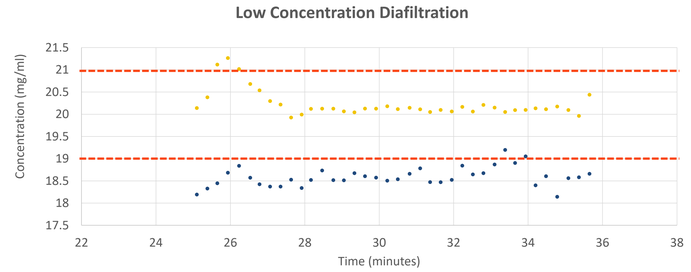

Figure 8. Low concentration diafiltration compared to desired concentration.

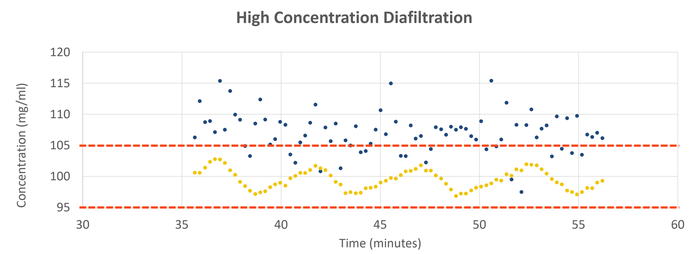

Figure 9. High concentration diafiltration compared to desired concentration.

Conclusion

By using concentration as a feedback loop to fully automate the TFF process instead of relying on the weight of balances, the processes were more accurate at achieving the desired concentration for each step. Implementing the FlowVPX System in-line allows for accurate process control without the manual intervention and human error that the balances introduce into the process.