Automated PAT-Driven TFF System Using In-Line Concentration Monitoring to Control the UF/DF Process

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Abstract

Development of effective, commercially viable process analytical technology (PAT) requires the interconnection of processing equipment and analytical tools with robust methods and reliable controls. This presentation will introduce the only PAT-automated TFF system that is controlled by real-time concentration measurement acquired via in-line variable pathlength spectroscopy. Real-time concentration measurement ensures consistent targeted concentration results, insensitive to the types of variation commonly seen in mass balance-only systems. Automated process control is demonstrated for various final concentration endpoints, suggesting a better solution for the UF/DF process with key advantages over existing methods.

Real-Time Process Management System

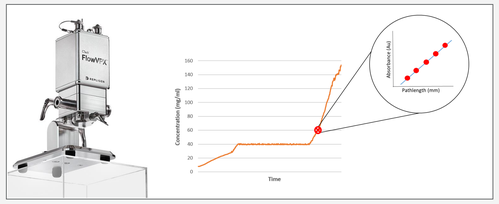
The Repligen real-time process management system (RPM) automation is driven by an in-line variable pathlength UV/Vis device, the CTech™ FlowVPX® System. The FlowVPX instrument is a variable pathlength technology (VPT) which utilizes the slope spectroscopy equation derived from Beer-Lambert law. The equation postulates that slope equals extinction coefficient multiplied by the concentration (m = εc). The FlowVPX System's search algorithm assigns a collection start value of 1 absorbance (1Au) and measures 5 to 10 decreasing pathlengths to generate a slope value. The adaptive nature of VPT allows for the FlowVPX to reliable generate concentration readings from 0.1mg/ml to 300mg/ml
at an EC of 1.5.

Beer-Lambert law:

$$A = \varepsilon l c$$

***Slope Spectroscopy*® equation:**

$$m = \varepsilon c$$

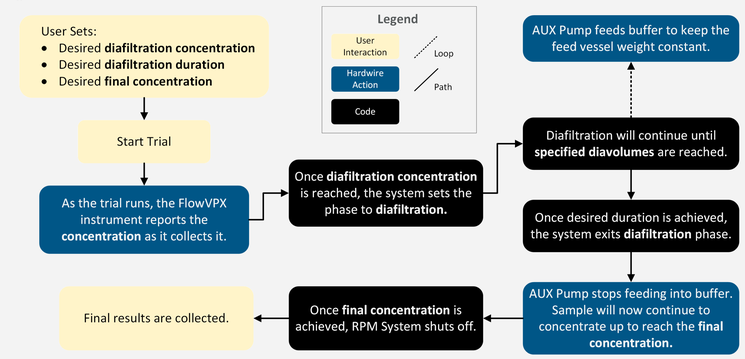


The new KrosFlo® KR2i RPM™ System utilizes Open Platform Communication – United Architecture (OPC-UA) to provide seamless PAT driven process control with two-way communication between each device within the system. Through innovative PAT and OPC-UA the RPM system provides reduced user input, greater process control and reliable process output.

TFF Automation

The objective of this study was to integrate an automated TFF process based on real-time concentration. The study included testing the efficacy of the systems automation via repeatably and post-run mixing outcomes. As well as a test of traditional mass balance vs conductivity and concentration-controlled processes. To achieve this, the FlowVPX variable pathlength spectrophotometer and KrosFlo® KR2i TFF System conducted a total of fifteen UF/DF processes. The UF/DF runs were conducted using BSA in replicates of three with starting concentrations of 5, 7.5 and 10 mg/ml. The final target concentrations for the processes were 50, 125 and 200 mg/ml. The first step was to ensure that each part of the KR2i TFF System was in communication with the FlowVPX System and its concentration readings, using the new integrated KrosFlo® RPM™ Software. A method then was developed to create parameters for each item to react. In this process, the endpoints for concentration stages of the process were being controlled through the FlowVPX concentration measurements.

Figure 2. TFF automation workflow



Data

Figure 3. Mass Balance vs In-line PAT UF/DF

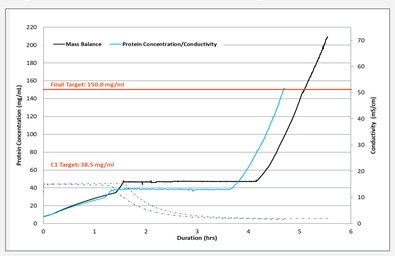


Table 1. RPM Final Concentration vs At-line UV/Vis

Bioprocess Step	FlowVPX System (g/l)	SoloVPE System, off-line (g/l)	Fixed Pathlength, off-line (g/l)
Initial	7.44	7.70	8.35
Diafiltration	38.80	39.58	39.94
Final	151.46	153.41	155.28

Figure 3 displays the outcome of our initial comparison testing of the mass balance vs conductivity and concentration-controlled process. The FlowVPX remained inline for both runs and recorded the real-time concentration but only the RPM run was controlled by these readings. The RPM system measurements were also compared to traditional UV/VIS devices to ensure consistency at 3 timepoints: Initial concentration, Diafiltration concentration, and end concentration. The repeatability testing generated 15 RPM graphs similar Figure 3. The results of the findings were expressed as percent deviation in final target points. The target specification limits for the RPM system are -0% / + 5% deviation.

Results

Repeatability testing found all 15 processes to be with in specification for target final concentration. The KR2i RPM System success rate for all process at varied start values and final concentration is a testament to the sensitivity and reliability of PAT based automation. The study found 12 out of the 15 processes to be within -0/+5 specification for target post run mixing concentration. With 100% of processes found to be within -1 /+ 6% for post run mixing. The key impacts to post run mixing variability within the UF/DF run came from the starting concentration, ending concentration and TMP. Higher flux and lower final pool volume runs were more susceptible to over concentrate. Whereas lower flux and high final pool volume runs were susceptible to under concentrate.

Table 2. Repeatability testing six-factor screening

A: Starting Concentration	B: Ending Concentration	C: Crossflow Rate	D: TMP	E: Retentate Hold Up	F: Starting Volume	Final Concentration Deviation	Post Run Mixed Concentration Deviation
5	50	6	8	5	2	0.08%	2.24%
10	200	6	18	10	2	0.15%	0.07%
7.5	125	9	13	7.5	2.5	0.18%	0.51%
10	200	6	18	5	2	0.52%	0.57%
5	200	12	18	5	3	0.35%	2.25%
5	200	6	8	5	3	0.05%	-0.72%
10	50	12	18	5	3	0.29%	0.92%
7.5	125	9	13	7.5	2.5	1.40%	0.98%
5	50	6	18	10	3	0.42%	1.96%
10	50	6	8	10	3	0.55%	0.72%
5	200	12	8	10	2	0.13%	0.55%
10	200	12	8	10	3	0.11%	-0.35%
5	50	12	18	10	2	0.80%	5.39%
7.5	125	9	13	7.5	2.5	0.14%	0.08%
10	50	12	8	5	2	0.08%	0.44%

The deviation found within the mass balance and PAT driven test can be attributed to a multiple factors. Variation in the final UF stage starting concentration, differences in accounting for hold up volume, and accurate scale taring all led to higher deviation in final concentration vs. the set point between the runs. The RPM system was set to end the diafiltration stage as soon as target conductivity was met vs. the mass balance which used a more conservative number of diavolumes to ensure buffer exchange was complete.

Conclusion

The KR2i RPM System provides an innovative solution to those looking to automate their TFF process as well as increase process control. The RPM system provides the first commercially viable PAT automated system through the incorporation of robust methods, OPC-UA communication and Variable Pathlength Technology. The study found the KR2i RPM System to reliably reach target final concentration as well as provide analytics for filter and process health. The real-time concentration measurement provided by the FlowVPX System ensures reliable concentration readings, mitigating the types of variation found in traditional mass balance setups. Future studies for the RPM system will be regarding post mixing optimization and novel modality testing.

Learn more at **repligen.com/kr2i-rpm**