PALL Biotech

Application Note

USD 3322

Monoclonal Antibody Concentration Monitoring of the Tangential Flow Filtration (TFF) Concentration Process by the mPath™ Index of Refraction Monitor

1 Introduction

In biopharmaceutical downstream manufacturing, product concentration is routinely performed with an ultrafiltration (UF) step. Depending on the downstream process design, UF may be implemented at multiple positions through the process, including before the chromatography steps, or at final formulation.

UF concentration is not considered a purification process. Relative impurity levels and the constitution of the buffer are not considered to change during the UF concentration step. The target protein product is concentrated by volume reduction through a tangential flow filtration (TFF) device. The conventional batch process often includes in-line volume monitor, transmembrane pressure (TMP) monitor, and in-process sampler and off-line laboratory analysis to secure the final product concentration.

In recent years, the biopharmaceutical industry has placed tremendous efforts on continuous downstream purification. To develop, operate and control a continuous UF concentration process, it is critical to track the biological concentration at a high sampling rate to ensure an accurate final product concentration.

The Pall mPath IoR concentration monitor offers a direct, accurate, and real-time in-line protein concentration monitoring technique through the measurement of refractive index of the protein-buffer system. The process analytical technology (PAT) can eliminate the necessity for sampling reducing potential sterility concerns and provides the product concentration in real-time, which enables quick responses to be made providing the opportunity to reduce process variation.

The Pall mPath IoR concentration monitor offers $^{1}/_{4}$ and $^{1}/_{16}$ in. interior diameter (ID) formats. Both formats have ferrules at the end of the flow cell, which is designed for Tri-Clamp♦ style sanitary tubing connection.

Here we show a real-world application of the Pall mPath IoR concentration monitor at a customer site. By connecting it to the single-use TFF system, the mPath IoR concentration monitor could provide reliable target protein concentration data in real time.

The part numbers of the Pall mPath IoR concentration monitor are listed in Table 1.

Table 1
Available formats of mPath IoR concentration monitor

Part Number	ID
MPATHRI1-FC16	$^{1}/_{16}$ in.
MPATHRI1-FC04	$^{1}/_{4}$ in.

Initially, a laboratory test was performed to obtain the pressure drop of the IoR concentration flow sensor. Deionized (DI) water was pumped through the IoR flow sensor at various flow rates. The pressure drop of the IoR flow sensor at different flow rates are plotted in Figure 1 and Figure 2.

Figure 1

Pressure-drop of DI water in the 1/4 in. ID mPath IoR concentration monitor (Note: pressure changes between lower flow rates were below the detection limit of the pressure sensor)

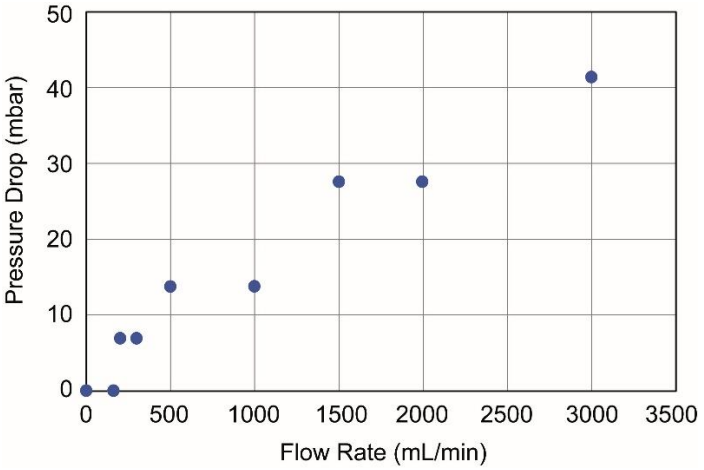
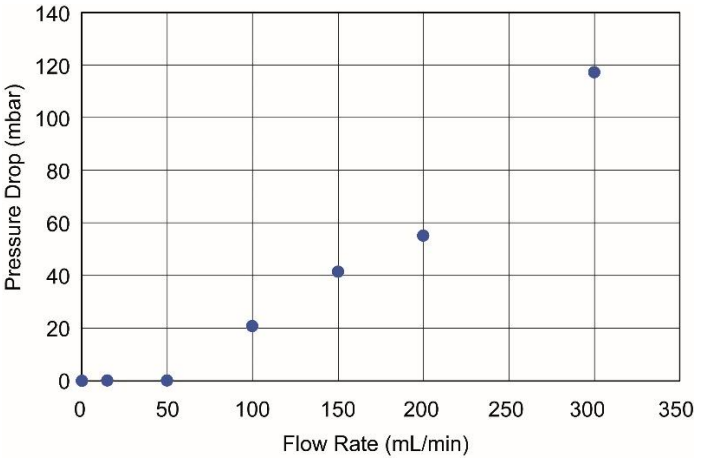


Figure 2

Pressure-drop of DI water in the 1/16 in. ID mPath IoR concentration monitor (Note: pressure changes between lower flow rates were below the detection limit of the pressure sensor)



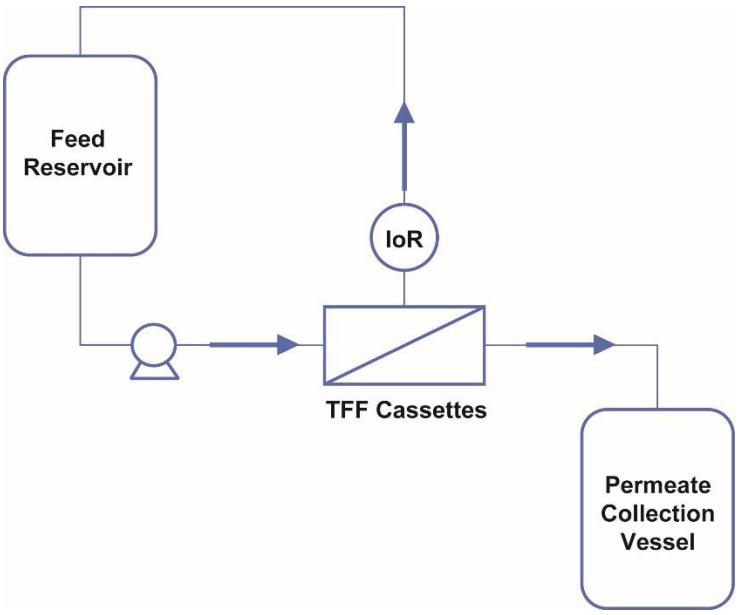
2 Experiment

At Biogen (Durham, NC, USA), the Pall mPath IoR concentration monitor was connected to a single-use TFF apparatus for a monoclonal antibody (mAb) UF concentration process. The UF concentration operation is to concentrate the purified mAb solution from 17 to 170 g/L.

Figure 3 displays the schematic of the UF concentration experiment with an mPath IoR concentration monitor mounted in the retentate flow path. During the UF concentration operation, the retentate was recirculated back to the feed reservoir and the permeate flowed to the permeate collection reservoir.

Figure 3

UF concentration experiment with real-time mAb concentration monitoring

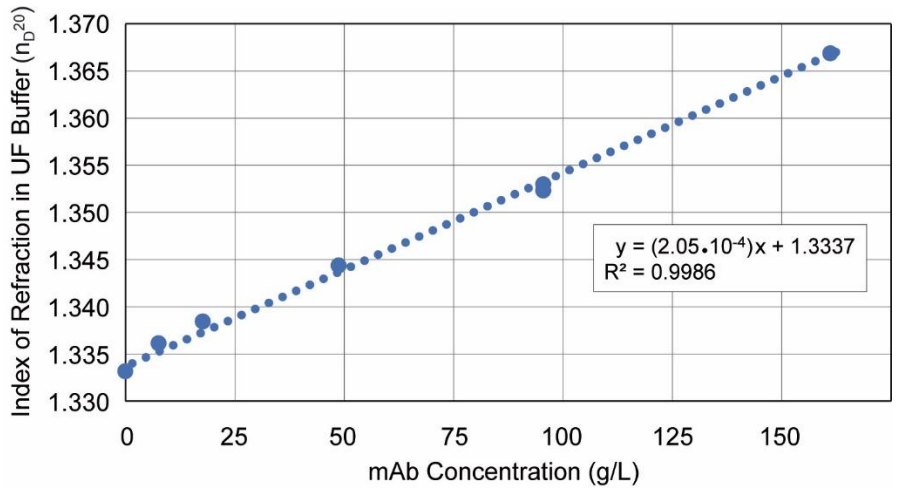


As a first step, prior to the process, a standard curve was established by linear regression between the refractive index and the mAb concentration at the pre-defined mAb concentration range. Six standard mAb solutions ranging from 0 to approximately 170 g/L were prepared in the UF buffer. The concentrations of the standard mAb solutions were measured off-line by a UV-Vis spectrophotometer. The refractive indexes of the standard solutions, typically expressed as n_D^{20} (D refers to 589 nm wavelength and 20 refers to 20 °C), were measured offline by the mPath IoR concentration monitor.

Figure 4 shows the linear standard curve of the mAb concentration and the IoR n_D^{20} data.

Figure 4

A six-point standard curve of the mAb concentration for the mPath IoR concentration monitor



During the UF process, the Pall mPath IoR concentration monitor was mounted near the retentate port of the TFF device. The UF concentration operation was continuously monitored for approximately 140 minutes with the test interrupted 4 times for liquid sample collection at the retentate port. The retentate samples were collected manually based on the real-time mAb concentration reading by mPath IoR concentration monitor at 25, 40, 80, and 160 g/L. The mAb concentrations were verified off-line with a UV-Vis spectrophotometer. The process was terminated when the retentate IoR mAb concentration reading reached 167.8 g/L.

Figure 5 demonstrates the real-time mAb concentration measured by mPath IoR concentration monitor during the UF concentration experiment (including the 4 waiting periods for the manual sampling collection). Times of manual sampling are noted with grey dots.

Figure 5

UF concentration test results: mAb concentration by mPath IoR concentration monitor

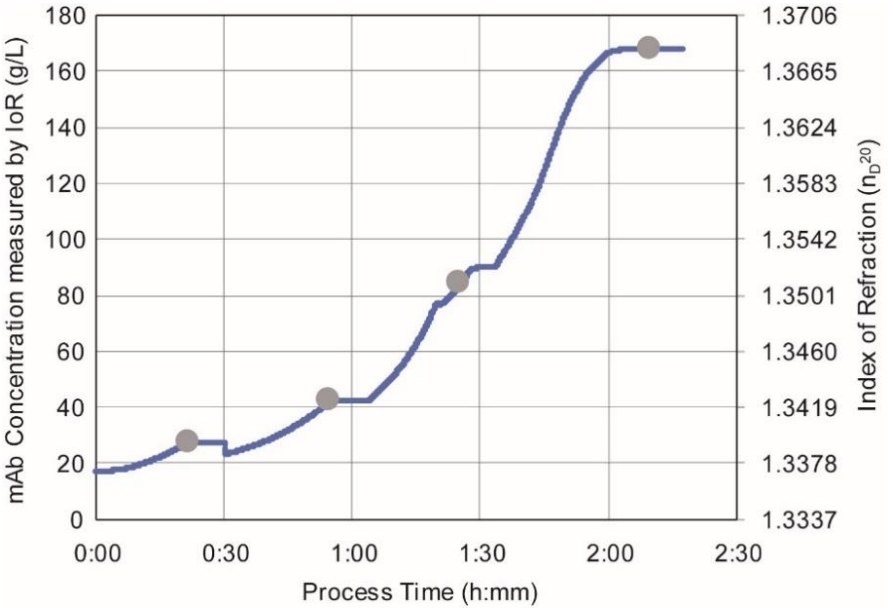


Table 2 compares the mAb concentration data acquired by the IoR concentration monitor (in-line) and the UV-Vis spectrophotometer (off-line).

Table 2

mAb concentration measured off-line by the UV-Vis spectrophotometer compared to the real-time IoR measurement by the mPath IoR concentration monitor

Sampling Time (min)	Index of Refraction (IoR n_D^{20})	mAb Concentration Measured by IoR (g/L)	mAb Concentration Measured by UV (g/L)	Difference (%)
21	1.3393	27.3	27.2	+0.37
54	1.3423	42.0	42.0	-0.12
84	1.3509	83.9	83.7	+0.24
129	1.3681	167.8	167.6	+0.12

3 Summary

The Pall mPath IoR concentration monitor provided a real-time, accurate, in-line determination of protein concentration during the UF mAb concentration operation. In this process, the Pall mPath IoR concentration monitor could accurately determine mAb concentration in real time for 140 minutes over the pre-defined product concentration range of 0 – 170 g/L.

Acknowledgements

In addition to data collection performed at Pall's Center of Excellence in Westborough, MA, USA, the authors would like to thank Biogen (Durham, NC, USA) for additional support.

PALL Biotech

Corporate Headquarters
Port Washington, NY, USA
+1.800.717.7255 toll free (USA)
+1.516.484.5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

Visit us on the Web at www.pall.com/biotech

Contact us at www.pall.com/contact

International Offices
Pall Corporation has offices and plants throughout the world in locations such as: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, the United Kingdom, the United States, and Venezuela. Distributors in all major industrial areas of the world. To locate the Pall office or distributor nearest you, visit **www. Pall.com/contact.**

Filtration. Separation. Solution.SM 12/19, GN 19.10124 USD 3322