BIOTECH

Application Note

USD 3475

mPath™ Index of Refractivity (IoR) Concentration Monitor for Chromatography Applications

1 Abstract

This document describes the capabilities of the mPath IoR (index of refractivity) concentration monitor for chromatography applications. The mPath IoR concentration monitor sensor is designed to have a wide linear detection range as well as the ability to track process changes in real time. These attributes make the mPath IoR sensor ideal for monitoring chromatographic processes. Here we show two chromatography applications for the mPath IoR sensor, 1) monitoring of product breakthrough and column saturation in real time, 2) monitoring elution concentration in real time.

2 Introduction

The process intensification of monoclonal antibody (mAb) manufacturing has seen significant development in recent years. Progress has been achieved on the optimization of the upstream process leading to an increase in product titers. Downstream processes have to accommodate for these increased titers and are in the need of further optimization. Process control plays a crucial role on this effort, which translates to an important need for accurate sensors which can monitor process parameters accurately and quickly during the downstream process, including chromatography. Typically, ultraviolet (UV) flow cells are integrated into chromatography skids to control and monitor chromatography performance. There are certain limitations to the UV sensors currently used, such as sensitivity and linearity. These limitations make it challenging to accurately load process feed to specific breakthrough percentages, necessitating a margin being taken on load capacity and subsequent underutilization of the resin. Additionally, during elution, it is common to achieve high product concentrations that saturate the UV sensors, making it impossible to monitor the elution concentration in real time.

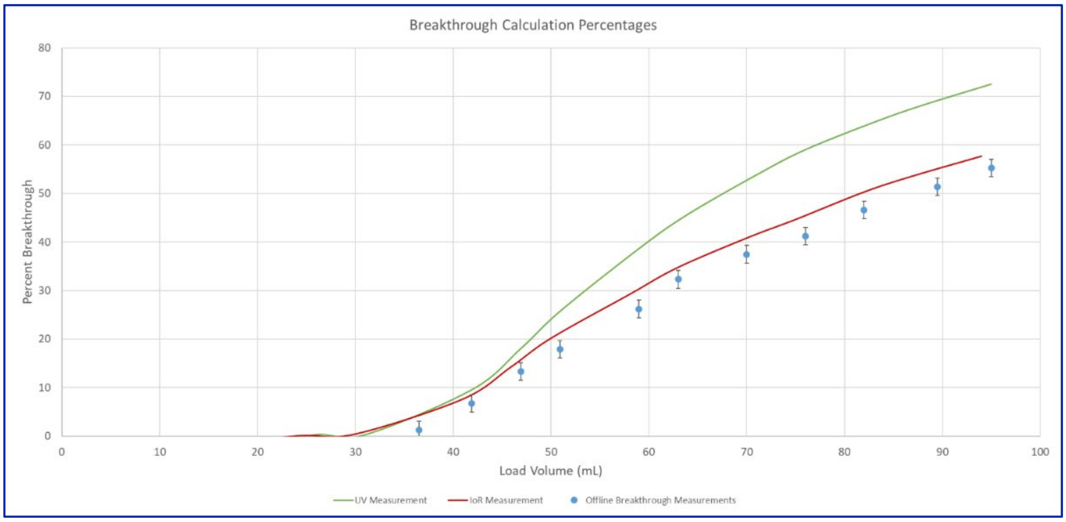
Here we describe the use of Pall's mPath IoR concentration monitor in these two applications: breakthrough and elution monitoring. We will show that the mPath IoR sensor enables loading to a consistent 10% breakthrough, with more accuracy and precision than traditional sensors. Additionally, we will present data showing accurate monitoring of the eluate concentration with the mPath IoR sensor.

3 Breakthrough Monitoring

The ability of the mPath IoR sensor to monitor the breakthrough of mAb from a Protein A column was benchmarked against a UV sensor. Breakthrough of mAb occurs during Protein A chromatography when the dynamic binding capacity is reached and unbound protein flows through the column. In this scenario, Protein A chromatography of mAbs, it is particularly difficult to monitor breakthrough of product from the column as the feedstock has an intrinsically high UV, which can already extend into the non-linear range of UV measurements.

Testing was performed on an ÄKTA◆ avant system with an integrated inline mPath IoR sensor, placed after the column (analogous to the UV sensor). A representative mAb feed with a concentration of 5.0 mg/mL was loaded onto a 5 mL column with a residence time of 1 minute. The output of the column, the flowthrough, was fractionated into 200 µl fractions throughout the loading process. The concentration of each fraction was then determined offline utilizing an immunoaffinity assay. The measurements from mPath IoR and UV sensors are compared to the absolute measurements performed by sampling and offline analysis. These concentration measurements returned the absolute breakthrough percentages throughout the loading step. The calculated breakthrough percentages by the mPath IoR and UV sensors were compared to the offline measurements obtained from the flowthrough concentration. This experiment was performed in triplicate and the results are shown in Figure 1.

Figure 1
Breakthrough percentages calculated by mPath IoR and UV sensors



It is seen in Figure 1 that the mPath IoR sensor is able to accurately monitor mAb breakthrough during loading. It is demonstrated that past 10% breakthrough the measurement from the UV sensor deviates significantly from the offline obtained measurements. This is most likely because the intrinsically high UV absorbance of the feedstock which may already push the UV sensor to the edge of its linear range.

10% breakthrough is a common measurement used in industry for Protein A column operation. A closer look is taken for the 10% breakthrough data previously obtained. The mass loaded at 10% breakthrough was calculated for both the mPath IoR and UV sensor methods and compared to the mass loaded calculated using the flowthrough offline concentrations measurements previously obtained, Figure 2, Table 1.

Figure 2
Mass loaded calculations to 10% breakthrough by UV and mPath IoR sensors

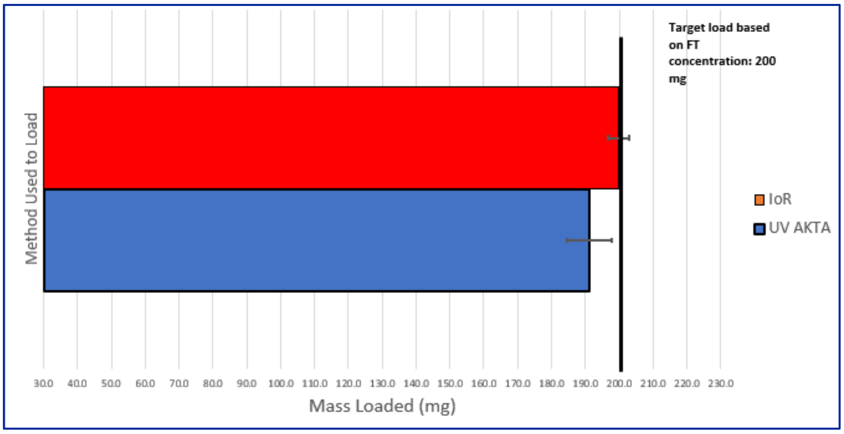


Table 1
Mass loaded measurements by mPath IoR and UV sensors and standard deviation

Method Used	Measured Mass Loaded (mg) Experiment 1	Measured Mass Loaded (mg) Experiment 2	Measured Mass Loaded (mg) Experiment 3	Average Mass Loaded (mg)	Standard Deviation
mPath IoR sensor	192	196	198	195	3
UV sensor	187	180	194	187	7

The data above shows that mPath IoR sensor is both more accurate and precise at loading to 10% breakthrough than the traditional UV sensor. The average mass loaded at 10% breakthrough based on the offline flowthrough measurements was 200 mg. An ideal sensor would be able to monitor the load with accuracy and precision and enable loading as close to 200 mg as possible. The mass loaded by the mPath IoR sensor was 195 mg with a standard deviation of 3, outperforming UV which had a calculated loaded mass of 187 mg and a standard deviation of 7.

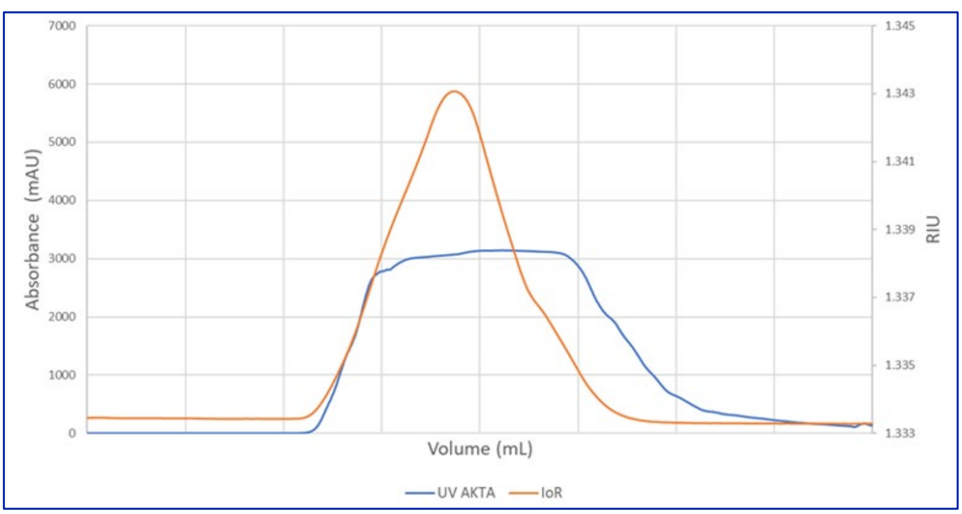
These results confirm that the mPath IoR sensor can be positioned to monitor breakthrough loading during Protein A, enabling for better overall process control.

Based on the previous positive results, the mPath IoR sensor was then used to monitor the loading of a Protein A column to 10% breakthrough. The loading was exclusively monitored by the mPath IoR sensor. As with the previous experiment the loading flowthrough was fractionated to determine the true breakthrough percentage. Results of this experiment showed that the column was successfully loaded to 10% breakthrough.

4 Protein A Elution Monitoring

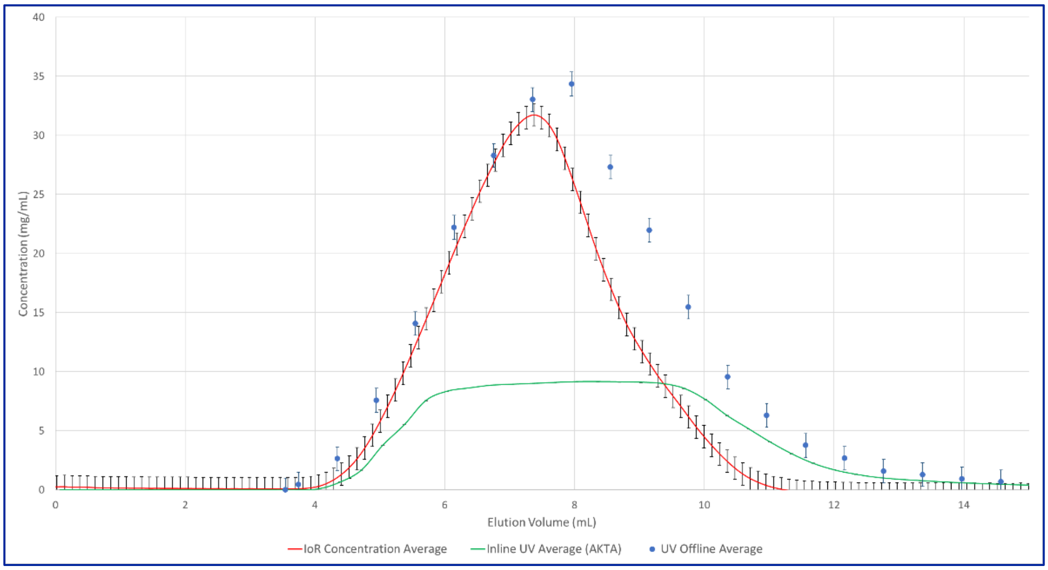
Taking advantage of the broad linear range of the mPath IoR sensor the elution concentration is monitored in real time. In this experiment a 5 mL Protein A column was loaded with a representative mAb, three wash steps were performed following the load, and the column was finally eluted with 100 mM acetic acid solution for three column volumes (total elution volume of 15 mL). The eluate was monitored with mPath IoR and UV sensors for comparison. The eluate was fractionated and measured offline by UV absorption to determine the absolute concentration of the elution. The signals obtained during elution with both methods are shown on the figure below (Figure 3).

Figure 3
Sensor signal obtained during Protein A elution with mPath IoR and UV sensors



The UV sensor suffers from signal saturation, while the mPath IoR sensor is able to capture the full elution peak profile. Taking advantage of this attribute, the theoretical elution concentration is calculated and compared to the measurements determined offline. In order to calculate the theoretical concentration, a simple concentration standard curve of the mAb used was built with both UV and mPath IoR signals. In the case of the mPath IoR sensor, the elution buffer is used as the background buffer for the standard curve. This experiment was performed in triplicate and the outcome is seen in Figure 4.

Figure 4
Elution concentration calculations using mPath IoR and UV sensors



It is apparent from the data that the mPath IoR sensor is able to monitor the concentration of Protein A eluate accurately. There is minimal deviation seen from the calculated mPath IoR concentration to the measurements obtained offline by UV absorption. Additionally, it is observed that the UV sensor is not able to calculate elution concentration as it saturates.

5 Conclusion

The mPath IoR sensors wide linear range and inline monitoring capabilities suit it to monitoring chromatography unit operations. The breakthrough of product from an overloaded column can be detected more accurately than with a UV sensor. This has applications for process intensification for both single and multi-column operation. In single column operation the sensor could be used to load columns to just beyond breakthrough, obviating the need to take a margin on the load capacity and subsequently driving capacity utilization. In multi column operation the sensor could be positioned in between load columns to monitor the process and act as a control strategy for loading capacity.

The sensor is also proven to successfully monitor elution concentration in real time, without experiencing signal saturation. This provides a pathway to monitor elution amount and column performance over time.

PALL CORPORATION

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

Pall Corporation has offices and plants throughout the world. To locate the Pall office or distributor nearest you, visit www.pall.com/contact.

The information provided in this literature was reviewed for accuracy at the time of publication. Product data may be subject to change without notice. For current information consult your local Pall distributor or contact Pall directly.

USD3475/ 21.07857
05/21