BIOTECH

Application Note

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mPath™ Index of Refractivity (IoR) Concentration Monitor: Protein Concentration Monitoring During Tangential Flow Filtration

Contents

1 Abstract

This document describes the capability of the mPath IoR (index of refractivity) concentration monitor for real-time analysis of tangential flow filtration (TFF). The mPath IoR concentration monitor includes sensors that are designed to have 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 product concentration monitoring during TFF. Here we show the application of the mPath IoR concentration monitor in two TFF settings: single pass (SPTFF) and a traditional final formulation via ultrafiltration/diafiltration/ultrafiltration (UF/DF/UF).

2 Introduction

Tangential flow filtration (TFF) is a common unit operation in bioprocessing. It is an efficient and rapid method for product concentration and buffer exchange. TFF relies on a pump to flow the sample solution containing drug substance across the surface of the filtration membrane. The portion that passes through the membrane is known as the permeate, while the remainder of the liquid, the retentate, is recirculated back to the feed reservoir.
An alternative to this approach, single pass TFF (SPTFF) performs concentration in a single pass through the device, not requiring circulation. This makes SPTFF highly suitable for process intensification through continuous processing and for labile molecules sensitive to shear.

For TFF and SPTFF monitoring the concentration of the product is crucial to process performance and efficiency. Current concentration monitoring techniques are mostly based on ultraviolet (UV) absorption. However, process streams commonly achieve concentration levels which exceed the linear range of available UV sensors. This does not allow product concentration to be measured directly inline. In these cases, the product must be sampled and measured offline. In the final formulation of a drug product this may necessitate over-concentration of the drug product, an offline concentration measurement and finally a dilution to the desired concentration. This approach adds processing time, additional resources and is overall less attractive economically.

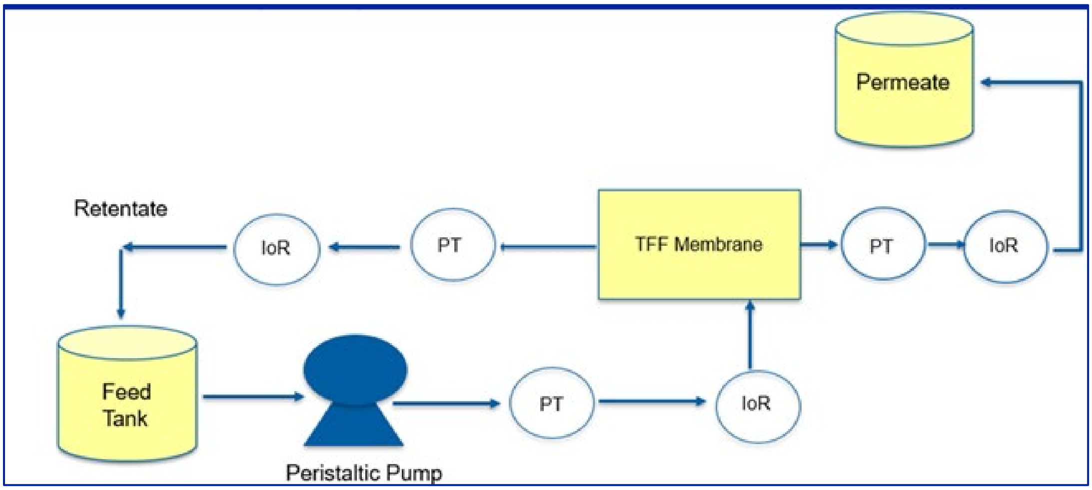
The details below describe the implementation of Pall's mPath IoR concentration monitor on two distinct TFF applications: UF/DF/UF and SPTFF. For UF/DF/UF applications, we show that the mPath IoR sensors enable accurate and robust determination of protein concentration, and buffer exchange completion in real time.
For SPTFF applications, we demonstrate the ability of the mPath IoR to be used as a process analytical control for continuous operation of SPTFF.

3 Using mPath IoR Sensors to Monitor mAb Final Formulation Ultrafiltration/Diafiltration/Ultrafiltration (UF/DF/UF)

Membrane based UF/DF/UF can be used to achieve desired mAb final formulation characterization. Here we mimic a UF/DF/UF final formulation process being monitored in real time utilizing mPath IoR sensors.
A representative mAb solution with a starting concentration of 5.8 mg/mL in 25 mM Tris, pH 6.5, 16 mS/cm was used. This mAb solution was processed to final concentration of 50 mg/mL and buffer exchanged into 16 mM histidine, a commonly used for final formulation buffer. This experiment was monitored with mPath IoR sensors placed on the feed, permeate and retentate streams. Pressure sensors were used for process control. The experimental setup schematic can be seen in Figure 1.

Figure 1

UF/DF experimental setup



High concentrations are commonly obtained during UF/DF/UF. Consequently, an inline UV sensor is not effective for concentration monitoring due to signal linearity limitations. Thus, it is common practice to obtain process samples to then measure offline with UV. In the figure below (Figure 2) we show the offline concentration measurements of the feed tank throughout the experiment. This is then used to compare against the concentration measurements obtained by mPath IoR sensor.

Figure 2

Feed tank offline concentration by UV sensor

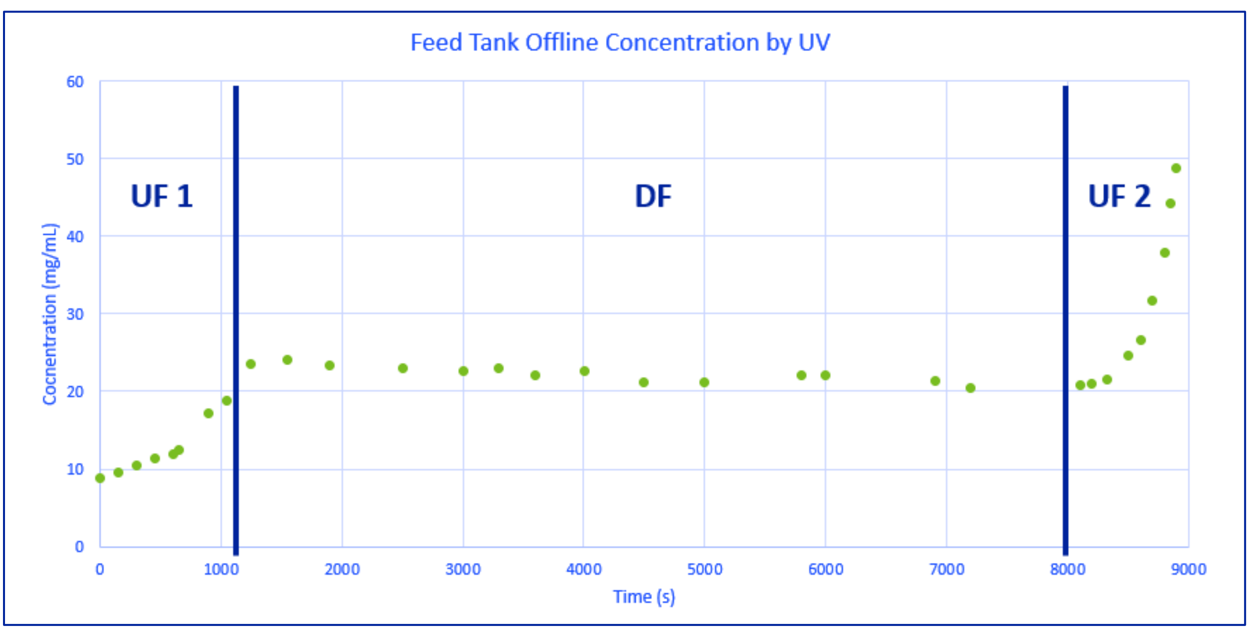
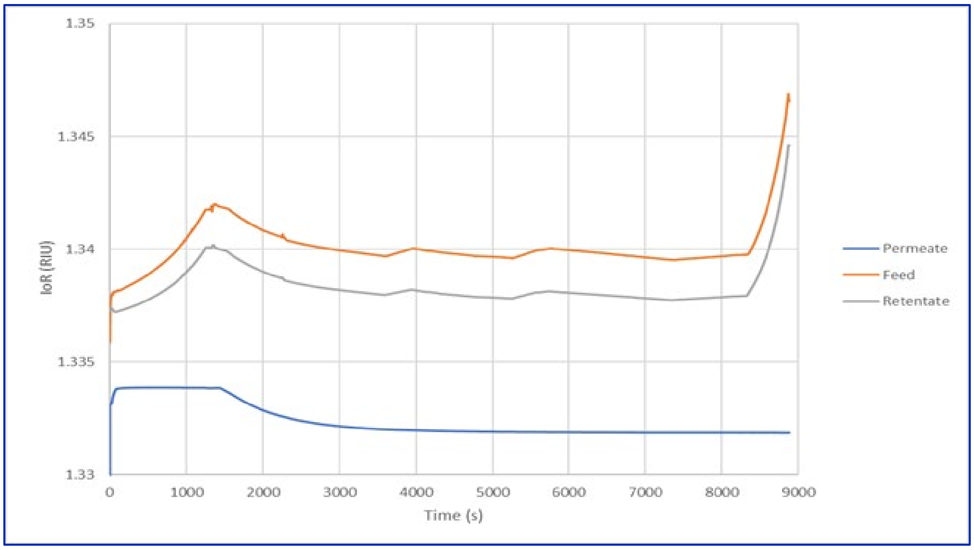


Figure 2 demonstrates the feed tank concentration profile during the experiment. There is an increase in concentration during both ultrafiltration steps and whereas a stable profile is seen during diafiltration. This profile is typical of UF/DF/UF. The signals obtained by the mPath IoR sensor for all three streams were analyzed and are shown in Figure 3.

Figure 3

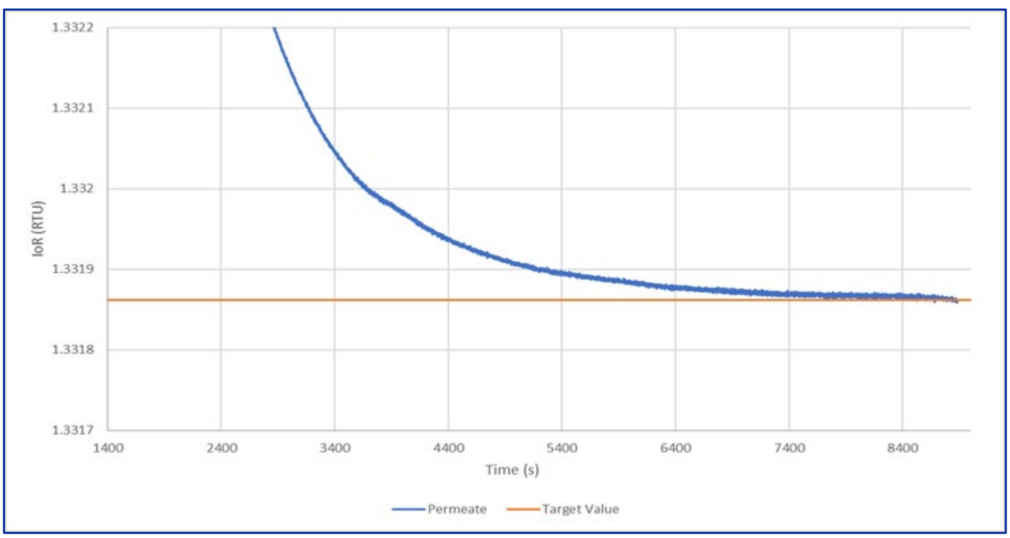
mPath IoR sensor signals during UF/DF



The ability of the mPath IoR sensor to differentiate between different buffer compositions allowed to obtain deeper process insight. The signal obtained from the permeate stream allows for characterization of the process buffer matrix in real time. During diafiltration this can be used to assess the progression of buffer exchange. The mPath IoR sensor permeate stream signal during diafiltration is seen in Figure 4.

Figure 4

mPath IoR sensor permeate stream during DF

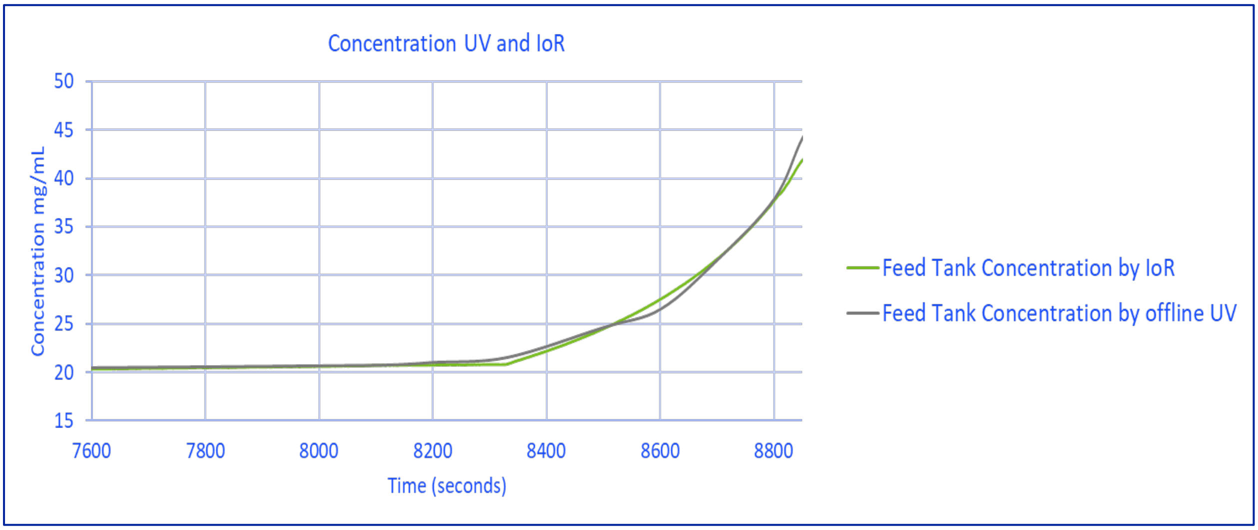


It is shown that the permeate stream will reach an asymptotic value when buffer exchange is completed. The index of refraction (IoR) value of the desired final formulation buffer can be obtained beforehand to define a set target value. This is shown to be a straightforward approach to identifying the completion of buffer exchange in real time. Ultimately buffer use is minimized, and overall process times are reduced.

Taking advantage of the broad linear range of the mPath IoR monitor, the feed stream concentration was calculated in real time. This measurement is calculated with the use of a previously built standard curve. This standard curve was generated utilizing the mAb of interest, in which the slope is a function of the molecule, while the y-intercept is the signal obtained from the buffer matrix. During UF/DF/UF, the process buffer matrix changes, making the concentration calculation from a previously generated standard curve a challenge. As the permeate stream allows monitoring of the buffer matrix, we can determine a real time y-intercept value. We then use this method to calculate the concentration of the feed stream as show on the Figure 5.

Figure 5

Concentration calculated by mPath IoR and offline UV sensors



The process concentration calculations utilizing the mPath IoR sensors are shown to accurately track the offline measurements by UV. Even at high concentration levels seen during the second ultrafiltration, measurements obtained with the use of the mPath IoR sensor are highly accurate. This method eliminates the need for offline concentration measurements and subsequent dilution procedures to achieve the desired concentration.

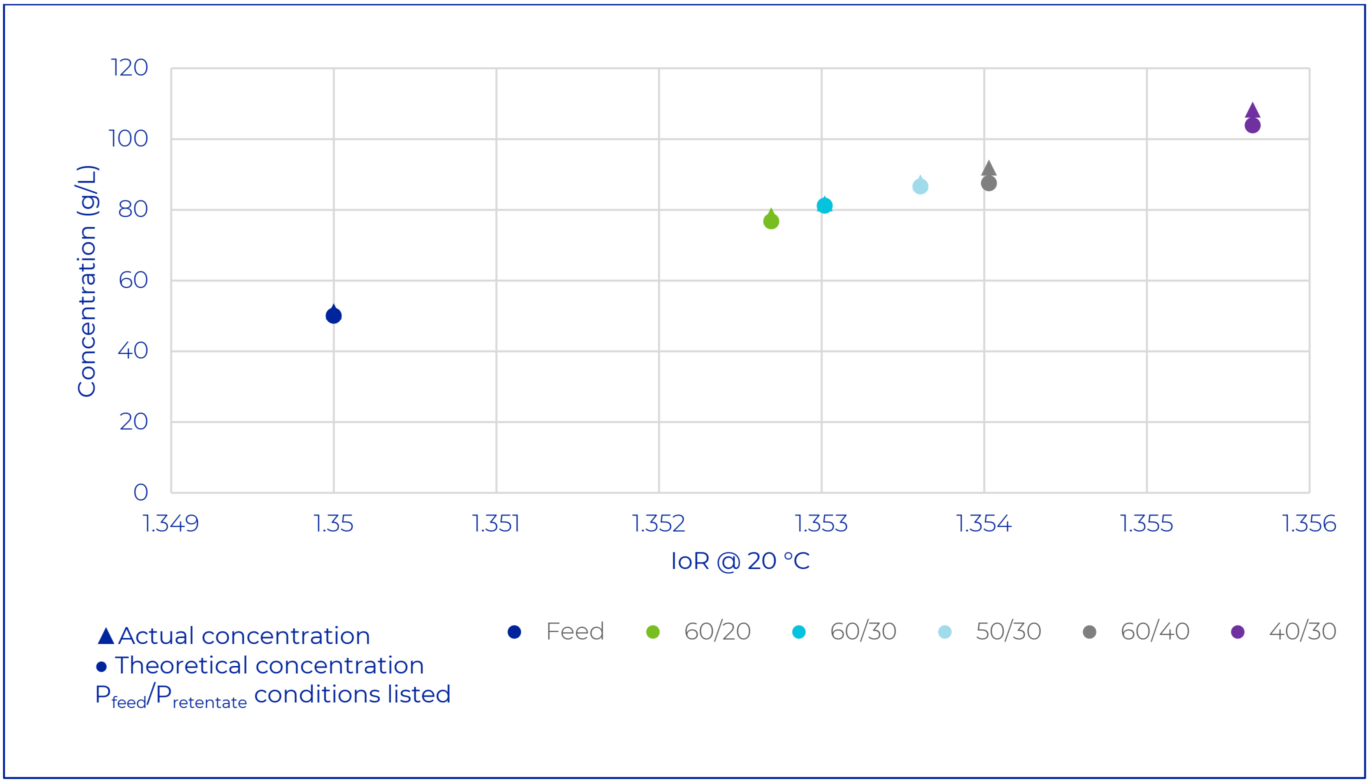
4 Characterization of Single-Pass Tangential Flow Filtration Configurations

A characterization study was performed using membrane based SPTFF configurations, which used both volumetric concentration factors (VCF) and the mPath IoR monitor. This study was performed using T-series TFF cassettes with Delta regenerated cellulose membranes and Omega™ polyethersulfone membranes. A representative bovine immunoglobin G (IgG) solution with a concentration of 50 mg/mL in 25 mM Tris, pH 7.5 was used. A total recycle configuration was used during this experiment, in which the retentate and permeate streams were directed back to the feed pool. This recirculation approach maintained a constant feed concentration throughout this portion of the experiment. The study was controlled by modulation of the feed and retentate pressures.

The mPath IoR sensor was used to evaluate different pressure parameters to determine the maximum concentration that can be achieved. Different pressure settings (P_{feed} / $P_{retentate}$) were established to determine the volumetric concentration factor (VCF) and theoretical concentration of the retentate. A standard curve was generated to calculate concentrations from the IoR signal obtained. The graph correlating the predicted concentration using VCF and the measured concentration using the mPath IoR is shown in Figure 6 below. The IoR values shown were acquired over a 5-minute period of stabilization for each pressure profile (P_{feed}/$P_{retentate}$ set points), which are indicated in the legend below.

Figure 6

Comparison of theoretical (using VCF) and measured (using mPath IoR sensor) concentration factors during excursion study of SPTFF



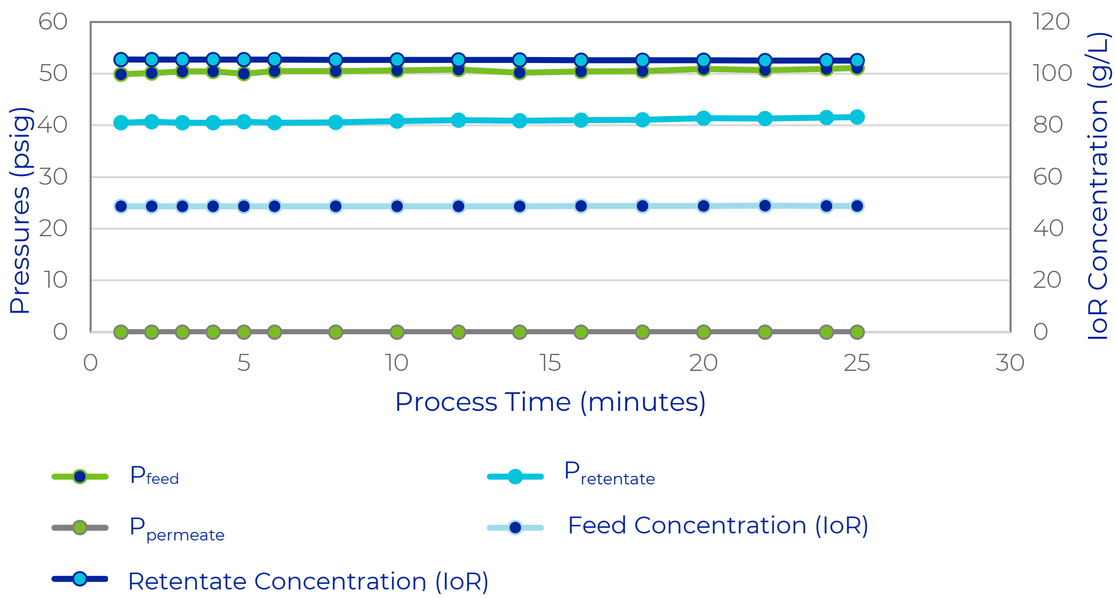
It is shown from the data in Figure 6 that the mPath IoR is a useful tool for process characterization. A strong correlation between the actual and theoretical concentration values is shown, with less than five percent difference across six distinct measurements. When establishing the highest concentrations that can be achieved with a given configuration, the mPath IoR can replace the need for offline sampling. This reduces time and resources needed for process optimization.

5 SPTFF Concentration Monitoring

Taking advantage of the broad linear range of the mPath IoR, the SPTFF operation was monitored in real time. For this experiment a representative IgG solution was used. The mPath IoR sensors were placed on the feed and retentate streams, aiming to monitor concentration and stability. At the same time process pressures, fluxes and volumetric concentration factor were recorded at intervals throughout the experiment. In order to calculate concentration from the mPath IoR sensor signal, the previously built IgG standard curve was used. The results of this experiment are seen in Figure 7.

Figure 7

Process pressures and mPath IoR sensor inline concentrations during SPTFF experiment



The data shown above demonstrates that the mPath IoR can be used to monitor process stability during SPTFF. The IoR signal can also be used to indicate fouling of the membrane as well and any other process deviations. This allows for fast detection of undesirable process changes.

6 Conclusion

The mPath IoR's wide linear range and inline monitoring capability makes it a suitable technology for monitoring of TFF unit operation. With the sensor's small footprint and ease of use, it can be readily incorporated for both batch and continuous configurations. In batch applications such as UF/DF/UF it allows the end user to monitor concentration in real time without the need of sampling and offline measurements. During SPTFF the mPath IoR sensor is shown to be a powerful tool for process configuration characterization. Finally, it is shown that during SPTFF the mPath IoR sensor is capable of monitoring process stability and detect process deviations.

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