Biotech

Application Note

USD 3324

Application of mPath™ Index of Refraction Monitor for Measurement of Monoclonal Antibody Concentration During Downstream Processing

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1 Introduction

During manufacturing of biologics, critical quality attributes (CQAs) of the product are assessed at different steps in the process. Some of the CQAs can be determined in-line with the appropriate sensors, while others are measured off-line by extracting samples and performing analysis. One of the most important CQAs is concentration of the product. Since most biologics are proteins, the current method of measuring concentration is using ultraviolet (UV) absorbance at 280 nm. The advantage of this method is that a UV flow cell can be placed in-line, and concentration monitored in real time. One challenge with UV is that typical detectors have a limited linear response of only up to 2 absorbance units (2 AU), which limits the limits protein concentration measurements to a maximum of 1.5 – 3.0 g/L, depending on extinction coefficient of the respective protein. The UV-based equipment capable of measuring concentration above this linear range, such as variable pathlength spectrophotometers, are expensive and have a large footprint, which imposes challenges for the laboratory or manufacturing floor, limiting their usefulness. Once protein concentration reaches high levels (above the linear response range of current UV sensors), without a variable pathlength spectrophotometer, process fluid will need to be sampled, diluted, and measured with an off-line analytical technique. All these additional steps increase the risk of bioburden introduction, errors in manual measurement, while adding on to the expenses and footprint of a bioprocessing facility.

A more convenient and cost effective method suitable for in-line monitoring of concentration with a wide linear range is measurement of index of refraction (IoR). Index of refraction technology enables direct, in-line measurement of fluid composition based on the refractive index of various fluid solutions. This technique can eliminate the need for sampling and off-line measurements.

We are demonstrating here that linearity in refractive index response covers protein concentrations typically encountered in monoclonal antibody (mAb) downstream purification process. Linearity, particularly at high concentrations (from ~4 to more than 60 g/L) will allow direct determination of concentration from IoR measurement. We show linearity for buffer conditions representative of steps in the process where protein concentration exceeds linear range for UV-based, single pathlength in-line measurement. The steps considered include: capture chromatography, polishing chromatography, and ultrafiltration.

Because refractive index is dependent on all components of a solution, the standard curves were created to demonstrate linearity and were performed in the buffers typically used during the purification process of a monoclonal antibody.

2 Materials and Methods

To demonstrate the efficacy of IoR across a broad range of conditions and product concentrations, a well characterized protein was diluted in buffers that matched various protein purification process steps.
A standard curve for each of those unit operations was designed to frame the protein concentrations typically seen in the respective unit operation. Each curve contains five points at 50, 75, 100, 125, and 150% of typical process protein concentrations for those unit operations in the respective buffers (Table 1).

To generate these standard curves, bovine serum albumin (BSA) was used as a surrogate for monoclonal antibody. Refractive index is a unitless measurement, typically expressed as n_D^{20} (D refers to 589 nm wavelength and 20 refers to 20 ^oC). The refractive index incremental change with concentration (dn/dc) for mAb is approximately 0.185/g, and the dn/dc for BSA is 0.190/g. With similar refractive index, the BSA will serve as a reasonable approximation of mAb for these IoR experiments.

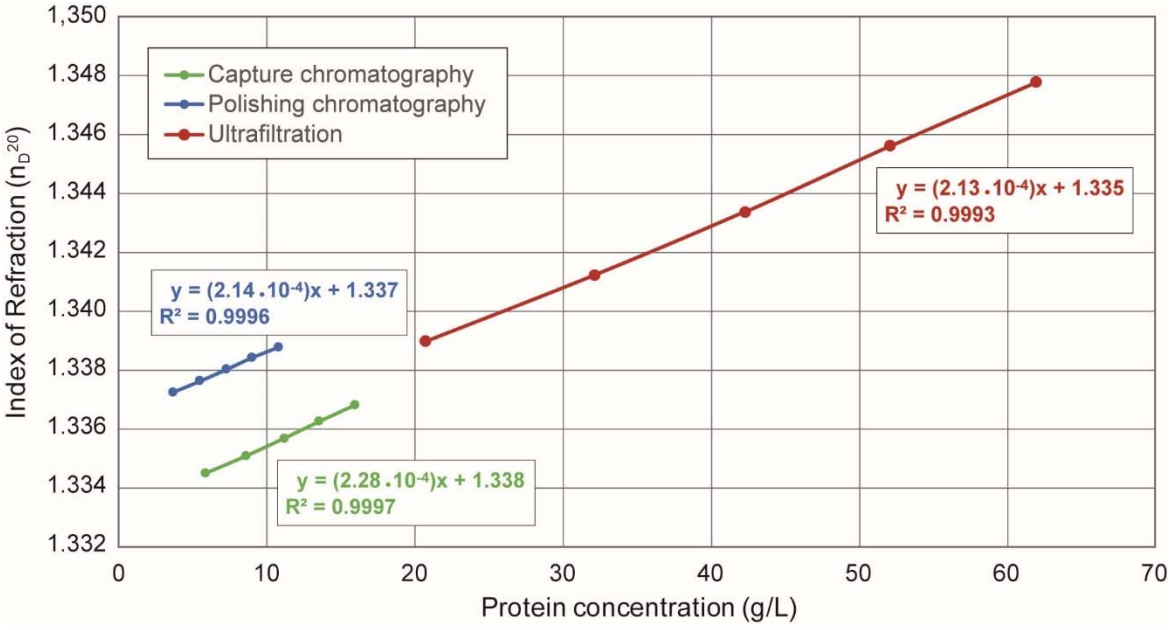
Table 1.
Typical mAb purification process steps with associated buffers and protein concentrations

Unit Operation	Process Buffer	Typical Concentration
Capture chromatography	50 mM acetate, pH 3.9	13.1 g/L
Polishing chromatography	50 mM Tris- acetate, pH 8.1	8.4 g/L
Ultrafiltration	10 mM histidine, 100 mM NaCl, pH 6.0	45.0 g/L

3 Results

Measurement of the refractive index of solutions of BSA at different concentrations were performed in various buffers, according to buffer conditions of the specific unit operations (Table 1). Generated standard curves are plotted, and linear regression equation of these standard curves is determined (Figure 1). Based on the linear regression equation for each condition (unit operation), the concentration of protein can be calculated from the index of refraction measurement.

Figure 1
The observed index of refraction signal (n_D^{20}) dependence on protein concentration in various buffer conditions used in a downstream monoclonal antibody purification process



Strong linearity, as seen in the determination coefficients in Figure 1 of these standard curves for all ranges of concentrations (from ~4 to more than 60 g/L) confirms that concentration can be calculated in real time directly from the signal of the mPath IoR monitor.

Protein concentration was mapped up to more than 60 g/L to establish a standard curve for in-line concentration determinations using index of refraction measurement. These concentrations span the range of protein concentrations encountered in a mAb purification process. Accordingly, it is shown that IoR in-line analytical technique can replace off-line measurements, thus eliminating product waste from sampling and potential error as a result of manual tasks.

The slopes of the standard curves were also calculated (Figure 1) and observed to all be within a tight range: $m = 2.18 \times 10^{-4} \pm 0.08 \times 10^{-4}$. This observation suggests that the variability in response of the mPath IoR sensor (incremental response) to protein concentration is very similar in all buffer compositions, and concentration factor changes can be predicted directly from the changes in index of refraction.

4 Conclusions

The mPath IoR in-line sensor can provide a real-time determination of protein concentration during all purification steps of a monoclonal antibody. Previously, such measurements were not available in real time for protein concentration higher than 1.5 – 3 g/L (varying with specific protein extinction coefficient) without using costly and bulky equipment. Standard curves are required to be generated in the appropriate buffer conditions prior to in-line use to calculate the product concentration in real time based on IoR signal. As seen in Figure 1, standard curves have very similar slopes with $m = 2.18 \times 10^{-4}$ ($\pm 0.08 \times 10^{-4}$), suggesting that the buffer condition simply changes the y-intercept, and variations in protein concentration will give similar variation in IoR signal. With a well-defined process and a pre-generated standard curve(s), this sensor can be implemented at various points in the process, and reliably measure product concentration over a wide linear range

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