High protein concentration UV sensing in bioprocess applications

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Introduction

Increased titers in cell culture, increased capacity of chromatography resins, and UF/DF operations with concentrations beyond 200 g/L in combination with stronger demands on process understanding/PAT put new demands on UV-Vis absorption sensing in bioprocess applications.

We present devices, concepts, and results that can address those challenges. By using UV flow cells with different path length in series, many different options are available and multiple needs can be addressed. The flow cells can be adapted to flow ranges from lab- to large-scale production equipment.

Flow cell design and properties

Figure 1 shows the design principle of the flow cells used. Two optical fibers are mounted in close proximity to each other creating a gap for measurement. The optics affect the flow only at a minor part or the cross section.

For lab-scale experiments a flow cell with 0.1 mm (peak elution) and 0.4 mm path length (PCC column loading) were used. Filtration experiments were performed with a modified process-scale flow cell. All flow cells use a similar design principle, but in different formats.

The scalability of the design was demonstrated by the wide flow range covered by the different flow cells. The maximum flow for the lab-scale and high-titer PCC flow cells were 150 and 75 mL/min, respectively. The process-scale flow cell with 8 mm i.d. could handle more than 16 L/min without producing significant back pressure.

A possible limitation could be that the flow exchange in the small gap is restricted. Under such circumstances, the concentration measured would not represent actual protein concentration, especially for fast processes and low flow and/or high viscosity conditions. However our results, including the linear gradient run in Figure 2, indicate that the approach is useful for many bioprocess related unit operations. More detailed studies are needed to more closely determine possible limitations.

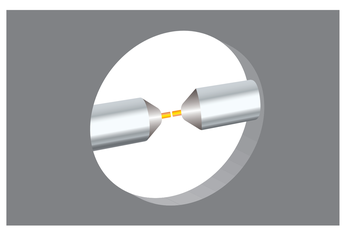

Fig 1. Design principle of the process-scale flow cell with 0.1 mm path length.

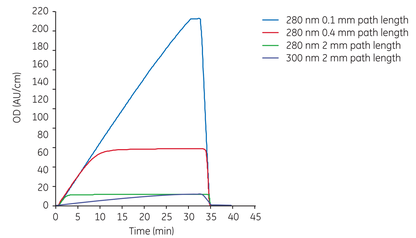

Fig 2. Linear gradient up to 7.7 g/L tryptophan in PBS buffer at pH 7.4 monitored with different path length/wavelength combinations.

Materials

A UV-900 monitor coupled to a prototype process- and lab-scale flow cell with 0.1 mm path length was used for most measurements. The PCC-related experiments were performed on an ÄKTA™ pure chromatography system using both a U9M/D UV detector with a 2 mm flow cell and a U9L single-wavelength detector with a 0.4 mm flow cell.

A mAb with 4 g/L concentration in HCCF solution was used for chromatography-related experiments.

An ÄKTA pure system was used for the PCC-related breakthrough curve. An ÄKTA flux filtration system was used for the filtration experiment.

Chromatography: peak elution

Figure 3 shows a typical affinity chromatography experiment. With a 0.1 mm flow cell, it was possible to get UV data within the dynamic range of the detector (200 OD), even though peak height corresponded to a mAb concentration around 85 mg/mL.

At 300 nm the peak shape appears adequate, but the peak height is not correct because the signal is close to saturation.

The use of very short path length flow cells enable peak maximum-based pooling criterion even for high titers/resin capacity.

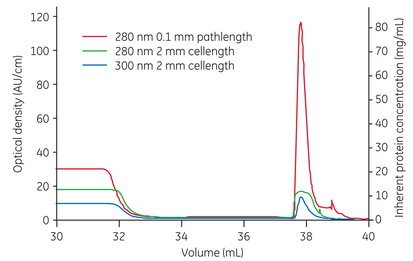

Fig 3. Typical affinity chromatogram showing wash and elution, using step gradient, for a mAb on a 1 mL HiTrap™ column prepacked with MabSelect SuRe™ resin. Around 60 mg mAb was loaded on the column at 0.5 mL/min.

Continuous chromatography: column loading

When measuring breakthrough in continuous chromatography, HCCF and impurities give a lot of background signal. Therefore, the ability to handle high titers is often required for accurate quantification of target protein breakthrough. Figure 4 displays a typical breakthrough curve recorded with different flow cells and wavelengths. It shows that the 0.4 mm flow cell gave the best conditions to quantify the breakthrough progression in this study.

The typical absorption spectra of a mAb in HCCF and a mAb in PBS buffer demonstrate that measurement at 300 nm is not satisfactory (Fig 5). For optimal quantification of protein breakthrough, the mAb peak absorption wavelength and a path length that results in around 1 to 1.5 AU response should be chosen.

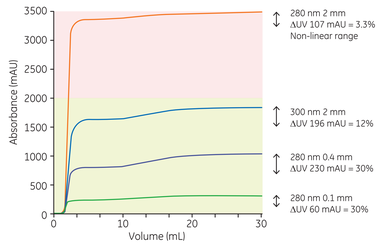

Fig 4. Breakthrough curves for mAb monitored with different path length/wavelength combinations. The sample was loaded to a 1 mL HiTrap column prepacked with MabSelect SuRe and run at 0.25 mL/min.

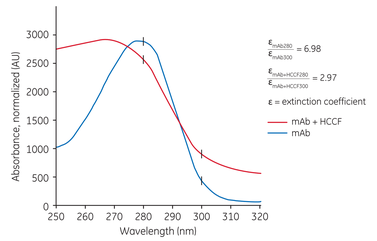

Fig 5. Normalized absorption spectrum for mAb in PBS buffer and mAb in HCCF.

Abbreviations

HCCF = host cell culture fluid; i.d. = inner diameter; OD = optical density; PAT = process analytical technologies;
PCC = periodic counter-current chromatography; UF/DF = ultrafiltration/diafiltration; UV-Vis = ultraviolet-visible spectrophotometry

Filtration

Very high concentrations of mAbs beyond 150 g/L can occur in the filtration step. Currently quantification is done off-line, often involving multiple dilution steps. This does not allow process control and is labor intensive and prone to errors.

Figure 6 shows a typical filtration run using a 0.1 mm path length process-scale UV cell at 280 nm wavelength. The perfusion flow was stopped at certain times. The data show that the on-line signal increases up to 190 OD, corresponding to 140 g/L mAb. The step function shows that the response is immediate. This demonstrates that there is a fast exchange of liquid in the small gap even at high concentration/viscosity. Hence short path length flow cells enable on-line monitoring and control.

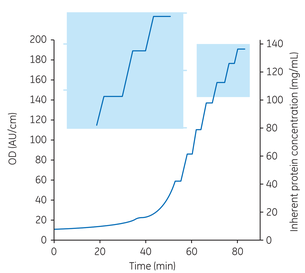

Fig 6. On-line UV signal for concentration of 5 g/L bovine γ-globuline using ÄKTA flux filtration system.

Conclusions

- UV flow cells with very short path length enable on-line process monitoring and control even in demanding applications with very high titers.
- The flow cells are useful in both conventional preparative chromatography, continuous chromatography, and UF/DF operations.
- Data indicates that mAb concentrations up to 150 g/L, corresponding to 200 AU/cm, could be quantified on-line, which would eliminate the need for external equipment and labor-intense sampling and dilutions. Further studies are required to confirm these indications.
- To adapt dynamic range, it is advantageous to modify the path length instead of the wavelength.
- Scalability to accommodate lab- to large-scale production is possible.

29216333 AA 06/2016