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Sensor Solutions

Application Note

In-line and Real-time Monitoring of Antibodies for Automated Purification

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Hightlights

- AugaOne™ can detect product breakthrough in-line and in real-time down to 2 µg/mL of antibodies.
- With the signal-to-noise ratio significantly higher than UV, AugaOne™ can operate in complex samples where the UV signal gets saturated.

Introduction

Therapeutic antibodies represent a promising avenue for treating a wide range of diseases and constitute a substantial portion of newly approved drugs. However, manufacturing these antibodies remains resource-intensive compared to traditional drug production, largely due to the complexity of the processes involved and the high expense of raw materials. Among the various steps in production, affinity purification is the most expensive, requiring significant effort to ensure the selective isolation of antibodies.

Over the years, several methods have been proposed to reduce the expenses associated with this step. One such approach is the use of multi-column systems to enhance column utilization and reduce product losses, though its effectiveness is limited by the absence of real-time monitoring capabilities. Another method involves using perfusion reactors with real-time release; however, traditional techniques for estimating column capacity are inadequate, as antibody concentrations vary with release times. Additionally, monoclonal antibody (mAb) binding capacity declines over time, by up to 40% after 100 cycles, leading to inconsistent results and unanticipated product losses even in the early stages.

This application note presents three examples demonstrating how localized surface plasmon resonance (LSPR) combined with the AugaOne™ sensor solution can be applied for real-time, in-line detection of product leakage and breakthrough during the affinity purification of IgG and domain antibodies (dAbs). This approach offers a more accurate and efficient solution for monitoring the purification process, improving overall yield and reducing production costs.

AugaOne™ for Affinity Purification

AugaOne™ is a versatile, stand-alone sensor system designed to seamlessly integrate with chromatography equipment, providing real-time, in-line data during the purification of therapeutic antibodies. Built on LSPR technology, AugaOne™ enables precise detection of product breakthrough with high sensitivity and specificity, accelerating downstream process development. Unlike traditional measurement methods, AugaOne™ does not require any sample pre-treatment, making it ideal for use in complex sample matrices where standard UV signals may become saturated.

With significantly higher signal-to-noise ratio compared to UV, AugaOne™ offers reliable performance even in challenging conditions, ensuring accurate monitoring of product concentration throughout the affinity purification process. The system's sensor chips can be tailored for specificity, functionalized with different ligands such as protein A and protein G for detecting IgG, and protein L for dAb fragments. This adaptability allows AugaOne™ to meet diverse analytical needs in the biomanufacturing of mAbs and dAbs.

By providing real-time insights into product leakage and breakthrough, AugaOne™ enhances process control, minimizes production variability, and supports more efficient use of chromatography columns. This makes it a valuable tool for reducing costs and improving yields in the manufacturing of therapeutic antibodies.

Materials and Method

The experimental setup for the AugaOne™ LSPR system (Fig. 1) consisted of a flow cell equipped with replaceable sensor chips. This flow cell was connected to an optical unit and positioned before the UV cell on an ÄKTA chromatography system (ÄKTA Pure™ 25, Äkta Purifier™, and Äkta Pilot™). The LSPR signals were monitored and recorded continuously in real-time, enabling precise detection of product concentration during the purification process.

Different IgG samples, provided by BioInvent were used in this study. Additionally, a human IgG1 was produced using a recombinant CHO (Chinese Hamster Ovary) cell line, provided by Cobra Biologics. All antibodies underwent purification using the AugaOne™ system integrated with the ÄKTA system, allowing for direct

comparison of in-line LSPR data with traditional UV measurements.

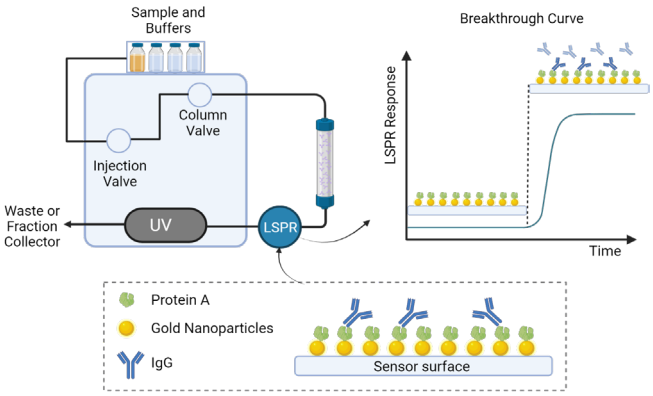

Figure 1 *Illustration of in-line detection of IgG during the loading phase in a purification set-up using a Protein A column to capture IgG from cell culture supernatants. An LSPR flow cell was connected after the affinity column to monitor the presence of IgG in the effluent during sample loading. The LSPR flow cell contains a replaceable sensor chip with gold nanostructures functionalized with Protein A. Selective binding of IgG to the surface when the IgG breaks through the column gives rise to a plasmon peak shift, recorded as an LSPR response and shown as a breakthrough curve.*

Three different experiments were performed:

- Experiment 1: Different IgG concentrations were used to validate the breakthrough detection.
- Experiment 2: Comparison of breakthrough detection in multicycle purification.
- Experiment 3: Detection of product leakage/ breakthrough using the AugaOne™ system.

Results

Experiment 1: Breakthrough Detection of Different Sample Concentrations

This study evaluated the effectiveness of using localized surface plasmon resonance (LSPR) for detecting IgG in cell culture supernatants following Protein A capture and comparing its performance with traditional UV detection. Two IgG concentrations were tested: 0.07 mg/mL and 1.5 mg/mL. LSPR demonstrated superior performance in detecting IgG breakthrough compared to UV detection, particularly in samples with complex backgrounds. At a concentration of 0.07 mg/mL, LSPR identified breakthrough at 88 minutes, while at 1.5 mg/mL, breakthrough was detected at 32 minutes. In contrast, the UV detection method struggled, with signals becoming saturated and unable to distinguish between background noise and true IgG signals at the lower concentration. One of the key advantages of LSPR was its higher signal-to-noise (S/N) ratio, especially for the lower concentration sample where UV detection was not effective. This allowed LSPR to provide earlier and more precise detection of IgG breakthrough, offering improved accuracy in monitoring the purification process. Additionally, the dynamic binding capacity (DBC) of the Protein A column was determined for both concentrations, with values of approximately 25 mg for the 1.5 mg/mL sample and 5 mg for the 0.07 mg/mL sample. These results align with the expected behavior of Protein A columns, where binding capacity decreases as antibody concentration drops. Overall, LSPR proved to be a more sensitive and accurate method for in-line detection of IgG compared to traditional UV detection, providing valuable real-time insights into the purification process. By changing Protein A to Protein L sensor chip, the breakthrough detection of dAb sample was equally achieved[1].

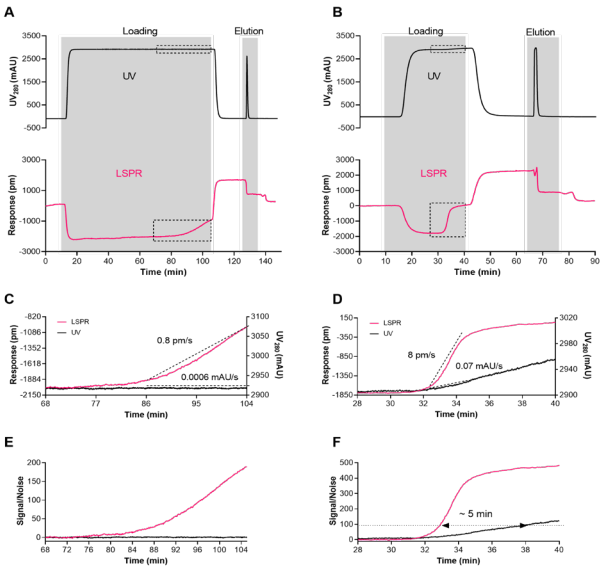

Figure 2 *(A) and (B) Real-time UV and LSPR signals from an IgG purification run using cell culture supernatant titers of 0.07 and 1.5 mg mL−1, respectively. (C) and (D) UV and LSPR breakthrough curves corresponding to the dashed squares in (A) and (B), respectively. (E) and (F) Data from UV and LSPR breakthrough curves in (A) and (B), respectively, plotted as signal-to-noise, calculated from the raw UV and LSPR signals normalized to zero at 68 min (E) and 28 min (F) and thereafter divided by the averaged noise level of the baseline before the product breakthrough.*

Experiment 2: Breakthrough Detection During Multicycle Purification

This study also included a continuous measurement experiment to simulate multi-column chromatography, extending the purification process over 15 hours and encompassing 8 loading and elution cycles. The goal was to further evaluate the performance of the LSPR sensor compared to UV detection during extended operation. Throughout the 15-hour experiment, the UV sensor reached its saturation limit at 3700 mAU, preventing accurate detection of product breakthrough. In contrast, the LSPR sensor effectively identified breakthrough events in all 8 cycles, even after each regeneration step using a pH 2.5 glycine buffer. This buffer allowed the LSPR signal to return to baseline after every cycle, facilitating continuous monitoring without signal interference. During the extended purification process, the LSPR sensor detected product breakthrough at a rate of 0.7 pm/s between 86 and 104 minutes. The UV sensor, however, remained saturated and was unable to register these changes. To evaluate the detection performance of both sensors, the S/N was used as a key metric. The LSPR system achieved a high S/N ratio of 200, indicating a clear distinction between signal and background noise, while the UV detection system struggled with a low S/N, failing to capture the subtle shifts associated with breakthrough events. Overall, the results from this extended experiment confirm that the LSPR sensor offers superior sensitivity and reliability during continuous, multi-cycle purification processes. Its ability to detect changes in product concentration in real-time, even under conditions that saturate traditional UV sensors, demonstrates its potential for enhancing efficiency in antibody purification workflows.

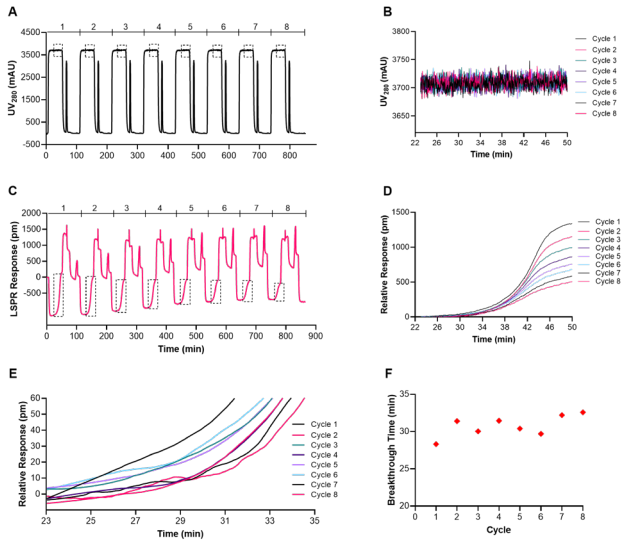

Figure 3 *(A) UV signals from eight subsequent cycles of IgG purification. Dashed rectangles mark the time range where product breakthrough was expected. (B) Zoomed-in UV signals from 23 to 50 min. (C) LSPR signals from eight subsequent cycles of IgG purification. Dashed rectangles mark the time range where product breakthrough was observed. (D) Breakthrough curves from eight cycles. The relative binding responses are the difference between the absolute responses and the baseline at 23 min of each cycle before the breakthrough occurs. (E) Zoomed-in area (from 23 to 35 min) of noise-reduced breakthrough curves. (F) Breakthrough time of each cycle. Breakthrough time was defined as breakthrough points at which relative responses reach thrvee times the noise or Limit of Detection (LOD), corresponding to 28 pm.*

Experiment 3: Detection of Unexpected Product Leakage/Breakthrough

In this experiment, the loaded sample amounts were intentionally kept below the expected column capacity to prevent breakthrough. However, in 3 out of 5 samples tested, the LSPR sensor detected unexpected IgG leakage between 90 and 110 minutes. The UV sensor, on the other hand, reached saturation in all cases and failed to detect these changes. Specifically, during the loading cycle of the hIgG1_01 sample (~0.3 mg/mL), LSPR detected breakthrough, prompting the collection of seven fractions between 40 and 130 minutes. Offline IgG quantification using LSPR confirmed an increase in IgG concentration from approximately 0.2 µg / mL to 7 µg /mL, validating the occurrence of leakage after 80 minutes. This result not only corroborated the presence of IgG leakage but also highlighted the sensitivity and selectivity of the LSPR sensor in detecting changes that were missed by the UV sensor.

The LSPR system's ability to identify subtle product losses in a scenario where no breakthrough

was anticipated underscores its effectiveness in continuous purification processes. This sensitivity is critical for optimizing column utilization and ensuring high product yields without unnecessary loss.

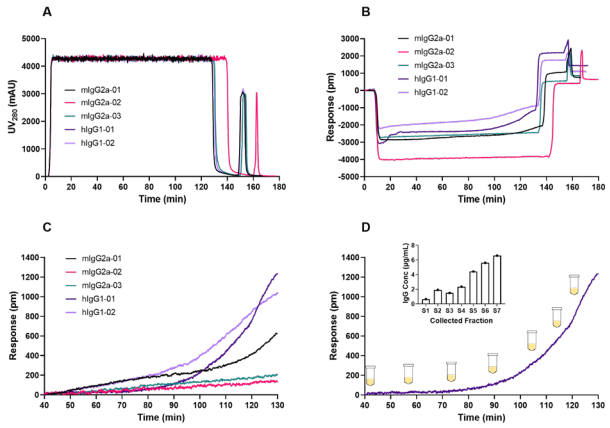

Figure 4 (A) Overlaid UV signals and (B) LSPR responses in affinity purification of three mouse IgG2a samples, mIgG2a_01 (0.19 mg mL−1), mIgG2a_02 (0.02 mg mL−1) and mIgG2a_03 (0.16 mg mL−1) and two human IgG1 samples, hIgG1_01 (0.28 mg mL−1) and hIgG1_02 (0.3 mg mL−1). Chromatography runs were performed using an ÄKTA Explore system with a flow rate of 3 mL min−1 using elution pH of 2.8. (C) Relative LSPR responses of five samples baseline-adjusted to zero at 40 min. (D) Breakthrough curve of the hIgG1L_01 sample and determined concentrations of seven fractions collected at different time points at the end of the loading phase.

Conclusion

This application note highlights the effectiveness of LSPR technology and the AugaOne™ system for real-time detection of unexpected breakthroughs and leakage in complex samples during antibody purification. The system demonstrated excellent performance across different purification scales, handling flow rates up to 200 mL/min, making it a powerful tool for process development and production intensification. By replacing lengthy off-line testing with rapid in-line measurements, AugaOne™ significantly accelerates purification workflows, offering results within seconds.

The LSPR sensor's ability to provide early and highly sensitive detection of product breakthrough and column leakage minimizes the risk of losing valuable samples. Additionally, the sensor surface can be regenerated in-line during the column stripping step, allowing it to be reused over multiple purification cycles. This study showed that the LSPR sensor maintained its sensitivity over eight continuous purification cycles, spanning 15 hours, making it suitable for long-term applications in biomanufacturing.

By offering precise, in-line monitoring during critical capture steps, LSPR sensor technology can significantly enhance the efficiency of automated processing, multicolumn chromatography, and continuous bioprocessing. This makes it an ideal solution for improving process control, reducing sample loss, and supporting the development of more efficient, scalable antibody production methods.

References

1. Tran T, Martinsson E, Gustavsson R, et al. Process integrated biosensors for real-time monitoring of antibodies for automated affinity purification. Analytical Methods. 2022;14(44):4555-4562. doi:10.1039/d2ay01567f