



In-line fiber optical sensor for detection of IgG aggregates in affinity chromatography

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ABSTRACT

Therapeutic monoclonal antibodies (mAbs) are critical for treatment of a wide range of diseases. Immunoglobulin G (IgG) is the most predominant form of mAb but is prone to aggregation during production. Detection and removal of IgG aggregates are time-consuming and laborious. Chromatography is central for purification of biopharmaceuticals in general and essential in the production of mAbs. Protein purification systems are usually equipped with detectors for monitoring pH, UV absorbance, and conductivity, to facilitate optimization and control of the purification process. However, specific in-line detection of the target products and contaminating species, such as aggregates, is currently not possible using convectional techniques. Here we show a novel fiber optical in-line sensor, based on localized surface plasmon resonance (LSPR), for specific detection of IgG and IgG aggregates during affinity chromatography. A flow cell with a Protein A sensor chip was connected to the outlet of the affinity column connected to three different chromatography systems operating at lab scale to pilot scale. Samples containing various IgG concentrations and aggregate contents were analyzed in-line during purification on a Protein A column using both pH gradient and isocratic elution. Because of avidity effects, IgG aggregates showed slower dissociation kinetics than monomers after binding to the sensor chips. Possibilities to detect aggregate concentrations below 1 % and difference in aggregate content smaller than 0.3 % between samples were demonstrated. In-line detection of aggregates can circumvent time-consuming off-line analysis and facilitate automation and process intensification.

1. Introduction

Since the approval of the first therapeutic monoclonal antibody (mAb) in 1986 [1], mAbs have become one of the primary treatment modalities for a wide range of diseases [2]. Production of mAbs and other biopharmaceuticals is highly complex and requires sophisticated and costly infrastructures. Process intensification and continuous manufacturing where multiple process steps are performed in series without interruptions can drastically reduce costs and environmental footprint [3–5], and facilitate production of biopharmaceuticals with high and consistent quality [6,7]. The development towards continuous biomanufacturing is highly encouraged by regulatory agencies, including the United States Food and Drug Administration (FDA) and

the European Medicines Agency (EMA), through the quality by design (QbD) initiative, the guideline on real-time release testing (RTRT), ICH Q13 guideline and process analytical technology (PAT) framework [8, 9]. However, the lack of real-time sensors for in-line monitoring of process parameters and critical quality attributes (CQA), such as product titer, host cell proteins, aggregates and other product variants and contaminants complicates process intensification and automation [10]. A broader implementation of continuous biomanufacturing thus requires new reliable process analytical technologies that can operate in-line and provide real-time information on CQAs in the different bioproduction steps.

The majority of the therapeutic mAbs in the market and under development are immunoglobulin G (IgG) [11]. IgGs are large

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macromolecules and tend to be prone to aggregation. Aggregation of IgG monomers into dimers and larger oligomers can have detrimental effects on the quality and therapeutic efficacy of the final product and can cause several adverse effects [12–15]. Aggregation is thus considered as a CQA in mAb production [16]. Varying process environments and conditions, such as temperature changes, agitation, anti-foaming agents, and osmolality considerably influence aggregation of mAbs and proteins in general [17–19]. The high ionic strength and low pH used in chromatography purification steps [20,21] and virus inactivation [22,23] can also contribute significantly to IgG aggregation. Analytical methods currently used in the biopharmaceutical industry to assess and quantify protein aggregate formation, such as size-exclusion chromatography (SEC) combined with ultraviolet (UV) or multi-angle light scattering (MALS) detectors, sedimentation velocity analytical ultracentrifugation (SV-AUC), and light obscuration (LO) are time-consuming, and require costly equipment that usually is located in separate quality control laboratories [8,24]. Raman spectroscopy shows potential for in situ detection of aggregates in lyophilized products [25,26]. However, in upstream bioprocess steps the complex sample matrix makes data analysis very challenging and the need for fast data sampling complicates the implementation in downstream purification steps.

Antibody purification is mainly based on chromatography. Affinity purification is typically used as a primary capture step followed by different polishing steps using anion/cation exchange or hydrophobic interaction chromatography [27]. Affinity capture using Protein A or G is commonly used as the first step in the downstream purification process of mAbs [28,29]. This purification step enables the separation of IgG from host cell proteins (HCPs), DNA, and many other contaminants present in the harvest supernatant. Aggregates formed in previous bioprocess steps or in the capture step due to low elution pH are usually present in the elution pool together with monomers and must typically be removed using other means of chromatography [28]. However, aggregates can also be separated and removed from monomers directly in the affinity capture step using a pH gradient elution [30]. Currently, UV detection is routinely used in the chromatography system and can detect monomers and aggregates when they are well separated during the elution but not when they are co-eluted in the same elution peak as the UV signal lacks selectivity.

We have recently demonstrated a nanoplasmonic fiber optical sensor based on localized surface plasmon resonance (LSPR) for on-line IgG titer measurements [31], breakthrough detection during affinity chromatography [32], and rapid at-line aggregate analysis [33]. Briefly, the detection system consists of a flow cell into which a replaceable sensor chip is inserted. The sensor chip is modified with gold nanostructures

with a defined surface chemistry and immobilized ligands for specific binding of a target analyte of interest. Here, the sensor chips were functionalized with Protein A for specific detection of IgG. IgG binding results in a time and concentration dependent wavelength shift of the LSPR band that can provide information on binding kinetics, affinities, and concentrations. Because of avidity effects, binding of IgG aggregates to the Protein A sensor chips results in distinctly different binding kinetics and LSPR shift compared to IgG monomers [33]. Detection of aggregate concentrations down to 30 µg/mL, corresponding to aggregate levels of 0.2 %, in just a few minutes were demonstrated [33].

Here we further explored the use of this technology for specific in-line detection of IgG monomers and aggregates during affinity chromatography. We connected the flow cell after the affinity column but before the UV detector in several different chromatography systems operating at different purification scales (Fig. 1). The sensor response was evaluated and compared to the UV signals and actual aggregate content in the eluent obtained by size exclusion chromatography (SEC). The LSPR sensor could detect difference in IgG aggregate content below 0.3 % in the eluant even when monomers and aggregates were co-eluted. In addition to on-line titer measurement and rapid at-line aggregate detection, this sensor strategy can thus facilitate in-line monitoring of CQAs during the affinity capture step, providing real-time information that can enable the optimization, intensification, and automation of affinity purification and removal of aggregates.

2. Materials and methods

2.1. Sensor chip preparation

Carboxylated sensor chips (provided by ArgusEye AB, Linköping, Sweden) were first activated using 20 µL of a mixture (v/v 1:1) containing 0.4 M of N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide (Sigma-Aldrich, St. Louis, MO, USA) and 0.1 M of N-Hydroxysuccinimide (NHS) (Sigma-Aldrich, St. Louis, MO, USA). The activation was carried out for 40 min and the sensor chips were then rinsed with MilliQ water (18.2 MΩ cm⁻¹). 20 µL of Protein A (Medicago AB, Uppsala, Sweden) at 0.5 mg/mL was added to the sensor chip surfaces and the coupling reaction was performed for 2 h. Unreacted carboxyl groups were deactivated for 30 min by using 20 µL of ethanolamine (Sigma-Aldrich, St. Louis, MO, USA) at 1 M, pH 8.5. The sensor chips were rinsed with MilliQ water and stored in phosphate buffer saline (PBS) containing 10 mM phosphate, 150 mM NaCl (Medicago AB, Uppsala, Sweden) until use.

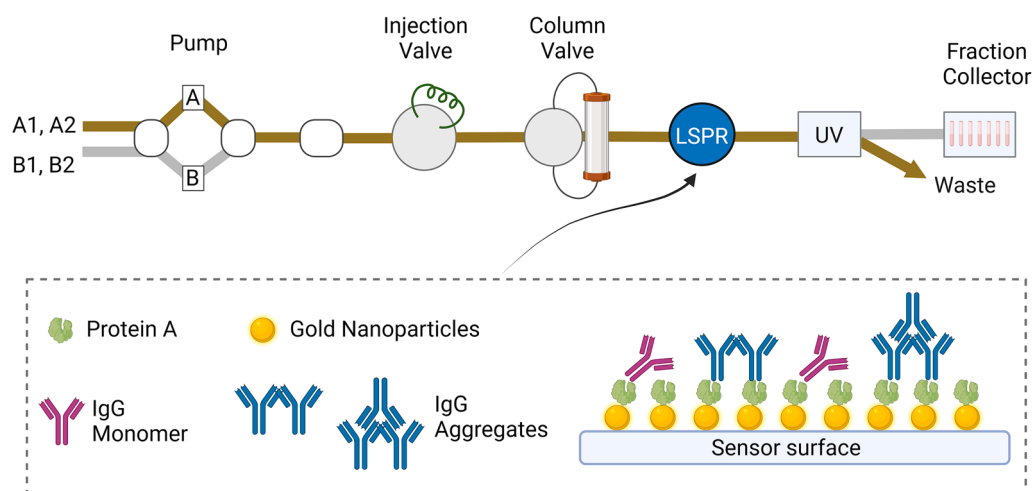


Fig. 1. Illustration of the in-line fiber optical LSPR sensor connected to a chromatography system for monitoring of the mAb capture step. Three different chromatography systems were utilized operating at different scales: ÄKTA pure™ 25, ÄKTA pure™ 150, and ÄKTA pilot™ 600.

2.2. Chromatography experiments

2.2.1. Affinity chromatography using pH gradient elution

Chromatography experiments using an ÄKTA pure™ 25 chromatography system (Cytiva, Marlborough, MA, USA) was conducted using a 2 mL MabSelect Prisma™ column (Cytiva) and clarified samples containing 2.6 mg/mL IgG (kindly provided by BioInvent AB, Lund, Sweden). The column was equilibrated using five column volumes (5CV) for 10 min of 10 mM phosphate buffer saline pH 7.4 (PBS) buffer before loading of the IgG samples for 32 min (~42 mg, equivalent to 21 mg/mL resin). Two washing steps using 20 mM phosphate, 0.5 M NaCl, pH 7.0 (5 CV) for 20 min and 50 mM citrate buffer, pH 5.0 (1 CV) for 4 min were performed after the sample loading. Elution was carried out using two different linear pH gradients, pH 4 to 3 and pH 4.5 to 3 using 50 mM citrate buffers (20 CV or 40 mL) for 80 min. A stripping step using 50 mM citrate buffer (2 CV) for 8 min, pH 2.3 followed by a CIP step using 0.5 M sodium hydroxide were used to regenerate the column. The column was finally re-equilibrated using 5 CV of PBS. A flow rate of 1 mL/min was used for equilibration and 0.5 mL/min was applied for other steps. A purification run using high loading (47 mg/mL resin) was also performed using similar chromatography parameters.

Purification using an ÄKTA pure™ 150 chromatography system (Cytiva) was conducted using a 30 mL MabSelect Prisma™ column, ~390 mg of IgG (or ~13 mg/mL resin for 28.6 min), and flow rates of 10 mL/min (for equilibration) and 5.23 mL/min for other steps. Total elution volume was 560 mL.

For purification using an ÄKTA pilot™ 600 chromatography system (Cytiva), 75 mL MabSelect Prisma™ column, ~2.4 g of IgG (or ~32 mg/mL resin for 71 min), flow rates of 25 mL/min (for equilibration) and 13 mL/min (for other steps) were applied. Elution was carried out with a linear pH gradient from pH 4.5 to pH 3 using 50 mM citrate buffer with a total elution of 1.44 L. The dynamic binding capacity (DBC) provided from the manufacturer for MabSelect Prisma is ~80 mg human IgG/mL resin at 6 min residence time and ~65 mg human IgG/mL resin at 4 min residence time.

2.2.2. Affinity chromatography using isocratic elution

For this set of experiments, the ÄKTA pure™ 25 chromatography system and a 1 mL MabSelect Prisma™ column were used. Equilibration (5 CV for 5 min) and washing (7 CV for 7 min) steps were carried out using PBS. Isocratic elution (12 CV or 12 mL for 12 min) was performed using citrate buffer 50 mM, pH 3.5 or 3.0. Stripping step was done using citrate buffer 50 mM, pH 2.5 (7 CV for 7 min). The column was re-equilibrated using PBS (5 CV for 5 min). Flow rate was set at 1 mL/min for the whole run. Standard mixtures with different aggregate percentages were prepared by mixing IgG aggregate and monomer stock solutions (4.5 mg/mL) (kindly provided by BioInvent, Lund, Sweden) and manually injected to the system through a 1 mL injection loop. These stock solutions were purified from the same IgG batch. To induce IgG aggregation of IgG in clarified cell supernatant 0.9 mg/mL (provided by Testa Center, Uppsala, Sweden) the samples were subject to heat-treatment. Samples were transferred into 50 mL Eppendorf tubes and incubated at 45 °C for 14 hours in an incubator, followed by another heating step in a water bath at 55 °C for 2 hours prior to purification on the ÄKTA pure™ 25 chromatography system as described above.

2.2.3. Size exclusion chromatography (SEC) analysis

To confirm the presence of aggregates in the elution pools, two different SEC systems were used, equipped with either a UV detector (SEC-UV) or a combined multi-angle static light scattering, refractive index and UV detector (SEC-MALS-RI-UV). Analytical size exclusion chromatography (SEC-UV) was performed using a Superdex 200 Increase 10/300 GL column (Cytiva) on an Agilent 1260 Infinity II Bio-inert LC system with OpenLab software (Agilent). Samples collected from the elution were injected at 100 µL, corresponding to ca 0–800 µg total mAb injected onto column. The mobile phase was 200 mM sodium

phosphate at pH 6.8. Separation was performed within 30 min at a flow rate of 0.8 mL/min with UV absorbance at 280 nm used for detection (reference signal at 360 nm). The full peak was integrated and split right before and after the monomer peak to calculate the relative abundance of high-Mw (aggregates), monomer, and low-Mw (fragments). The SEC-MALS-RI-UV included an Agilent HPLC 1100 system with a multi-angle light scattering, refractive index, and UV detectors from Wyatt Technology (Santa Barbara, CA, USA). Refractive index changes were measured differentially with a GaAs laser at a wavelength of 690 nm, and UV absorbance was measured with the diode array detector at 280 nm. A Superdex 200 column was used for the separation of monomers and aggregates. The flowrate was set at 1 mL/min and 100 µL of samples were injected for all measurements.

3. Results and discussion

3.1. In-line monitoring of Protein A chromatography using different pH gradients

Affinity purification of mAbs using Protein A resins is most often carried out using isocratic elution, which results in good recovery but is typically not efficient in removal of aggregates. The use of various elution additives [34] or pH gradients [30] can facilitate separation and removal of IgG aggregates already in the affinity capture step, which can simplify the following polishing steps. However, even with a good separation, the low concentrations of aggregates typically result in a very low signal from the UV detector. Because of the high sensitivity of the LSPR sensor and possibilities to distinguish monomers and aggregates in at-line setups, we first explored the possibility to detect aggregates in clarified cell culture samples during affinity chromatography using elution with pH gradients (Fig. 2A).

In the clarified samples, cells and other larger particulate matter were removed by centrifugation, leaving a complex protein-rich sample matrix. During the loading phase (zone 2 in Fig. 2A,C), the LSPR response showed a substantial and rapid negative baseline shift due to the strong color of the sample. Even though minute amount of product escaping the column during the loading phase can be detected using the LSPR sensor, the baseline remained stable during the loading phase with no signs of column breakthrough [32]. The baseline shifted back to approximately the initial baseline during the washing step. The small increase in the baseline level after the loading phase is likely a result of the different refractive index properties between the equilibration and washing buffers. Moreover, there were some signal fluctuations during the two washing steps (zone 3 in Fig. 2A and C) when the ÄKTA pumps were purged for faster buffer exchange, but this did not affect the overall performance of the sensor.

As expected, the UV detector gave a distinct response during the elution phase with a shoulder corresponding to the aggregate fraction when using a linear pH gradient decreasing from pH 4 to pH 3 (Fig. 2B). An even better aggregate separation was obtained when running the pH gradient from pH 4.5 to pH 3 (Fig. 2D). The aggregates elute later than monomers due to the avidity effects caused by aggregates binding to multiple protein A molecules in the resin [30]. In the LSPR sensor, higher binding response was observed for pH gradient 4 to 3 (Fig. 2B) compared to pH gradient 4.5 to 3. Because of the faster transition to low pH conditions for the pH 4–3 gradient, a higher concentration of monomers was obtained at the beginning of the elution, resulting in a higher LSPR signal.

We have previously shown that the Protein A sensor chips used here can enable detection of IgG concentrations down to 2 µg/mL [32]. As a consequence of the high sensitivity of the LSPR sensor, a more pronounced increase in the LSPR signal compared to the corresponding UV signal was obtained during the early stages of the elution phase (Fig. S1, Supplementary Materials). During the first couple of minutes of the elution, low concentrations of monomers (86 %) and fragments (15 %) were eluted, resulting in a slight (~20 mAU) increase in the UV

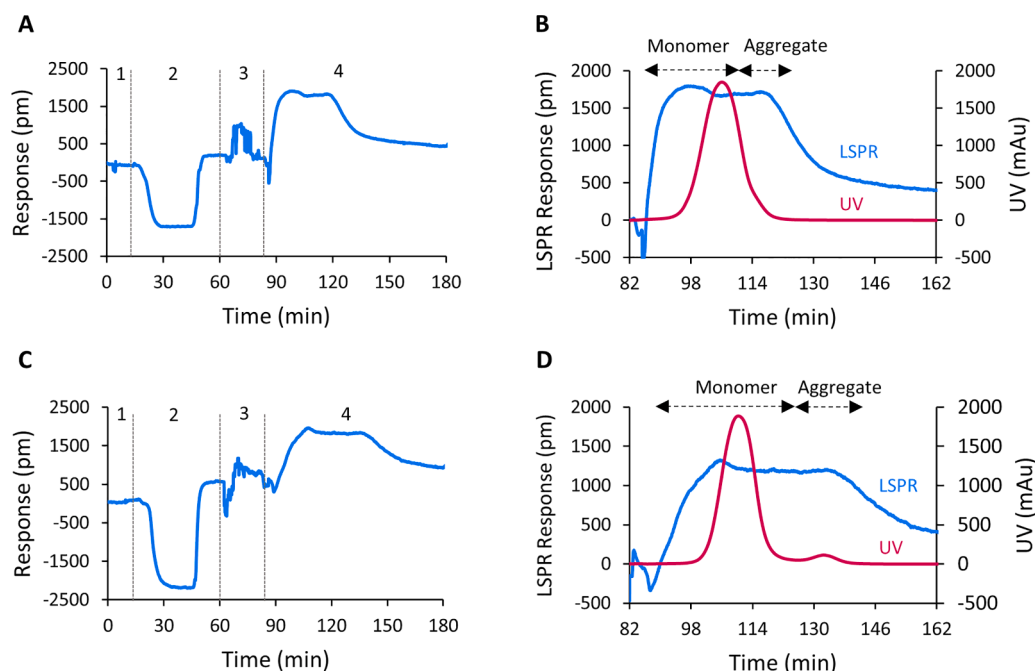


Fig. 2. In-line monitoring of protein A chromatography using linear pH gradients during elution from pH 4 to pH 3 (A,B) and pH 4.5 to pH 3 (C,D). (A,C) LSPR sensorgrams of two purification runs including (1) equilibration, (2) sample loading, (3) washing, and (4) elution. (B,D) Extracted and normalized elution profiles from (A) and (C), respectively.

absorbance. However, because of the high affinity interaction between IgG and Protein A on the sensor chip, a sharp increase (~ 1500 pm) in the LSPR response was observed. The LSPR signal continued to increase until the low pH of the elution buffer caused the IgG monomers to start dissociating from the sensor chip about midway through the elution phase. However, the elution of the aggregate fraction resulted in a distinct increase in the LSPR sensor response (Fig. 2B,D). We recently reported that pH-dependent avidity affects can be used for sensitive detection of IgG aggregates using an at-line LSPR sensor setup by diluting samples containing varying amount of IgG aggregates in buffers with slightly acidic pH values [33]. The apparent dissociation constant was almost one order of magnitude lower for aggregates compared to monomers in the pH range 3.8–4. The distinct increase in the LSPR response observed here thus indicates the possibilities to detect aggregates in-line during affinity chromatography. To further challenge the sensor system, aggregate detection at higher sample loading, corresponding to 47 mg of IgG per mL resin, was investigated (Fig. S2, Supplementary Materials). Because of the high protein concentration, the UV-sensor was saturated during the elution phase and could not detect the early onset of the aggregate elution. The higher loading resulted in a slight decrease in the resolution between the LSPR signals of aggregates and monomers. However, a clear shift in the LSPR signal was still seen during early stage of the elution of aggregates, further confirming that aggregates can be detected in-line in real time.

3.2. Comparison of UV and LSPR signals in relation to the content of fragments, monomers, and aggregates

To facilitate the analysis of the monomer and aggregate fractions and their contributions to the LSPR sensor response, we continued to investigate the performance of the in-line sensor using two scale-up chromatography setups; ÄKTA pureTM 150 and ÄKTA pilotTM 600 systems, enabling the use of larger columns and higher flow rates and thus better means to analyze the content of the elution pool. The elution was performed using a linear pH gradient from pH 4.5 to pH 3 in both cases. Forty fractions were collected along the elution phase and were analyzed by off-line SEC-UV to quantify the relative amounts of

fragments, monomers, and aggregates. The LSPR sensor response was comparable to that when using the smaller ÄKTA pureTM 25 chromatography system. As discussed above, due to the high but pH dependent affinity of the Protein A sensor chip, the LSPR sensor picked up the onset of the IgG elution earlier than the UV sensor and showed dissociation of monomers with decreasing pH. The increase in the LSPR sensor response, indicating aggregate elution, aligned with the increase in the UV response. A small shoulder in the UV peak at about 46 min (Fig. 3A) and 50 min (Fig. 3B) in the chromatograms from the ÄKTA pureTM 150 and ÄKTA pilotTM 600 systems, respectively, indicated co-eluted aggregates, which coincided with a more pronounced increase in the LSPR response. The peak area of fragments, monomers, and aggregates obtained from off-line SEC-HPLC analysis confirmed the presence of aggregates in these fractions. Factors such as elution pH and gradient rate, sample loading, and relative abundances of fragments, monomer and aggregates, influenced the IgG binding profile, resulting in a unique and information-rich LSPR sensor response that can provide data that complement the UV signal and facilitate decision making and fraction collection.

3.3. LSPR-based detection of aggregates using isocratic elution

Because isocratic elution is robust and fast, it is the most common choice for the capture step. However, aggregates are then not separated from monomers and are hidden in the monomer peak and cannot typically be detected in-line by the UV detector. In contrast, since the LSPR response is related to the relative affinities/avidities for monomers and aggregates at specific pH values, detection of aggregates from monomers is also possible under isocratic conditions. Five standard samples with varying percentages of aggregates (0.1 % to 14 %) in PBS were prepared and loaded on a MabSelectTM PrismA column and eluted isocratically at pH 3.0 (Fig. 4). Remarkably, the LSPR sensor could clearly differentiate these samples based on the IgG dissociation rates during the elution phase. Higher aggregate percentages resulted in a distinctly slower dissociation of IgG from the sensor surfaces. Since IgG aggregates show higher avidity to the protein A sensor chip, aggregates dissociate slower from the sensor surface than monomers. These findings correlated well

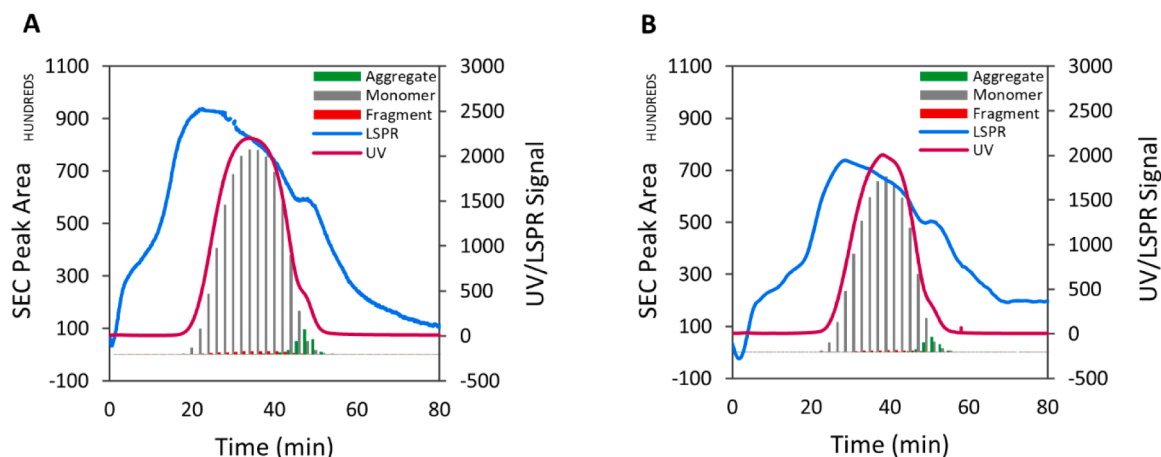


Fig. 3. LSPR and UV in-line monitoring of the elution phase in protein A chromatography using (A) ÄKTA pure 150 and (B) ÄKTA pilot 600 chromatography systems. The bars indicate SEC-UV peak area of fragments, monomers, and aggregates, obtained from off-line SEC-HPLC analysis from forty fractions collected during the elution.

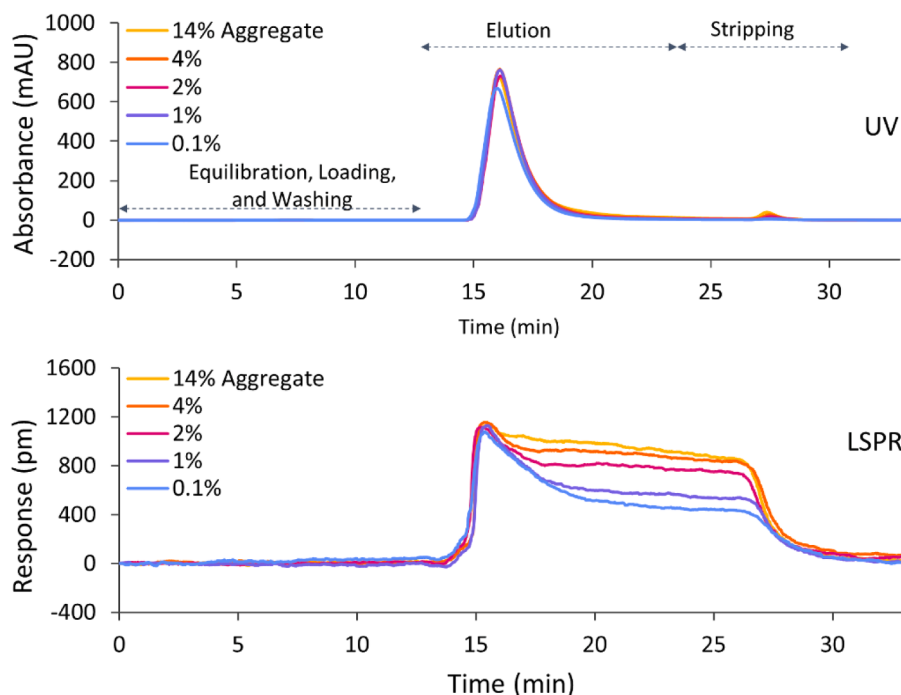


Fig. 4. Comparison of LSPR and UV in Protein A chromatography using isocratic elution (pH 3.0) and five IgG standard samples (4.5 mg IgG) containing different amounts of aggregates. Aggregate content was confirmed using off-line SEC-MALS-RI-UV analysis of the elution peaks.

with our previous work showing avidity effects of aggregates when binding to protein A immobilized on the sensor chips [33]. IgG aggregates have also been observed to bind with higher apparent affinity to immobilized Fc receptors because of the presence of multiple interaction sites on IgG dimers and oligomers [35,36]. During the column stripping step, the LSPR sensor chip was efficiently regenerated and the signal returned to baseline (Fig. 4). To further verify these findings, we subjected clarified cell culture samples to a mild heat treatment to generate samples with identical composition and properties but different IgG aggregate profiles. The monomer, dimer and oligomer peaks in the SEC chromatogram of the heat treated and untreated samples showed different intensities but were eluted at similar retention times (Fig. 5), indicating that they shared similar properties but different aggregate content. Three replicate affinity chromatography runs were performed for each sample with the same amount of IgG (~12 mg) (Fig. 5A).

Elution was done isocratically at pH 3.0. As discussed above, due to the strong color of the clarified cell culture samples the sensor baseline level decreased during the loading phase and then shifted back to the original baseline level after the washing step. The magnitude of the negative baseline shift during loading was slightly different for the two samples because of the effect of the heat treatment on the sample color. Noticeable differences in the LSPR sensor response during the elution phase of these two samples were observed (Fig. 5A, inset), while the corresponding UV peaks were identical (Fig. S3, Supplemental Materials). The heat-treated samples showed significantly higher binding response at 40 min and slower dissociation rate, indicating higher aggregate content compared to the untreated samples (Fig. 5B). The data are mean values of three replicates and includes dimers and small oligomers. The result correlated well with the experiments using monomer samples spiked with different amounts of aggregates shown in

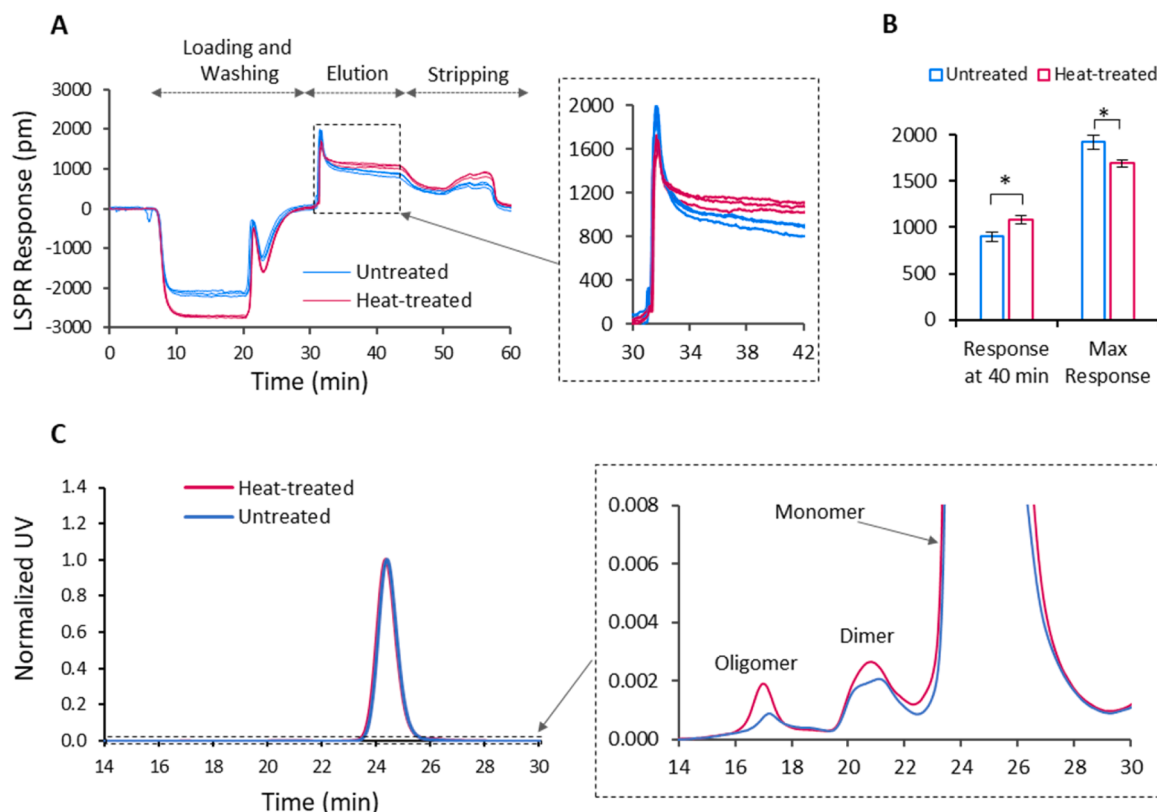


Fig. 5. In-line monitoring during protein A chromatography of heat stressed samples. (A) LSPR chromatograms of samples before (blue) and after heat treatment (red) to induce aggregate formation ($N = 3$). (B) Comparison of the two sample sets with respect to LSPR response at 40 min and maximum response (*, $P < 0.05$, t -test, $N = 3$). (C) Representative SEC chromatograms of elution pools obtained by off-line analysis using SEC-MALS-RI-UV. Intensities/UV absorbance of the monomer peaks are normalized.

Fig. 4. The aggregate content in the two samples were determined using SEC-MALS-RI-UV analysis of the elution pools for the untreated and heat-treated and were found to be as low as 0.6 ± 0.1 % and 0.87 ± 0.06 %, respectively (Fig. 5C). The difference in aggregate content between the two sample sets was thus less than 0.3 %. The performance of the LSPR sensor for in-line detection of aggregates during affinity purification is consequently on par with the corresponding LSPR-based at-line sensor setup [33]. To our knowledge, the sensitivity and specificity reported here would be challenging to accomplish using other techniques for in-line aggregate monitoring and indicate a unique potential to use LSPR for real-time monitoring of CQAs. Moreover, the distinct differences in the LSPR response for different elution conditions and aggregate content indicates a potential to quantify aggregates in-line.

4. Conclusions

Real-time monitoring of CQAs is central for successful bioprocess intensification and automation to increase productivity and product quality while lowering production costs. In-line sensors that can detect specific impurities early in downstream chromatography steps can greatly accelerate this development. The nanoplasmonic fiber optical LSPR-based in-line sensor described here can detect both monomers and IgG aggregates with high sensitivity during column loading and elution in affinity column chromatography of mAbs. We demonstrate detection of aggregates during both gradient and isocratic elution, also in cases where the UV detector was unable to differentiate them from the main product. Differences in aggregate content as low as 0.3 % resulted in significant differences in the LSPR response during the elution phase. This study was carried out in small lab scale chromatography systems up to pilot scale. We anticipate that combining LSPR sensors with UV detectors for in-line monitoring during affinity chromatography can

significantly improve real-time monitoring of this process step by enabling rapid detection of aggregates with high sensitivity when using both pH gradients and conventional isocratic elution. The potential benefits of LSPR also extend to other down-stream unit operations, including the polishing step, underscoring its relevance for future investigations. Moreover, because of the possibilities to immobilize ligands for other target analytes, the LSPR sensor can facilitate specific detection of other relevant products and impurities. Combined with the flexible flow cell design, this sensor technology can thus offer new possibilities to implement real-time monitoring of CQAs throughout the process train at industrially relevant conditions.

CRediT authorship contribution statement

Thuy Tran: Writing – original draft, Methodology, Investigation, Formal analysis. **Robert Gustavsson:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Erik Martinsson:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Filip Bergqvist:** Writing – review & editing, Resources, Methodology, Investigation. **Andreas Axen:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition. **Ingemar Lundström:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Carl-Fredrik Mandenius:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Daniel Aili:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal

relationships which may be considered as potential competing interests: Thuy Tran reports financial support was provided by Marie Skłodowska-Curie grant agreement No. 841,373. Daniel Aili reports financial support was provided by The Swedish Innovation Agency (VINNOVA), grant numbers 2016–04120 and 2019–00130. Daniel Aili reports a relationship with ArgusEye AB that includes: board membership and equity or stocks. Erik Martinsson reports a relationship with ArgusEye AB that includes: equity or stocks. Ingemar Lundström reports a relationship with ArgusEye AB that includes: equity or stocks. Carl-Fredrik Mandenius reports a relationship with ArgusEye AB that includes: equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.chroma.2024.465129](https://doi.org/10.1016/j.chroma.2024.465129).

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