

Real-Time Monitoring and Control of the Load Phase of a Protein A Capture Step

Matthias Rüdert, Nina Brestrich, Laura Rolinger, Jürgen Hubbuch

Karlsruhe Institute of Technology, Karlsruhe, Germany; telephone: +49 721 608 42557;
fax: +49 721 608 46240; e-mail: juergen.hubbuch@kit.edu

ABSTRACT: The load phase in preparative Protein A capture steps is commonly not controlled in real-time. The load volume is generally based on an offline quantification of the monoclonal antibody (mAb) prior to loading and on a conservative column capacity determined by resin-life time studies. While this results in a reduced productivity in batch mode, the bottleneck of suitable real-time analytics has to be overcome in order to enable continuous mAb purification. In this study, Partial Least Squares Regression (PLS) modeling on UV/Vis absorption spectra was applied to quantify mAb in the effluent of a Protein A capture step during the load phase. A PLS model based on several breakthrough curves with variable mAb titers in the HCCF was successfully calibrated. The PLS model predicted the mAb concentrations in the effluent of a validation experiment with a root mean square error (RMSE) of 0.06 mg/mL. The information was applied to automatically terminate the load phase, when a product breakthrough of 1.5 mg/mL was reached. In a second part of the study, the sensitivity of the method was further increased by only considering small mAb concentrations in the calibration and by subtracting an impurity background signal. The resulting PLS model exhibited a RMSE of prediction of 0.01 mg/mL and was successfully applied to terminate the load phase, when a product breakthrough of 0.15 mg/mL was achieved. The proposed method has hence potential for the real-time monitoring and control of capture steps at large scale production. This might enhance the resin capacity utilization, eliminate time-consuming offline analytics, and contribute to the realization of continuous processing.

Biotechnol. Bioeng. 2017;114: 368–373.

© 2016 The Authors. *Biotechnology and Bioengineering* published by Wiley Periodicals, Inc.

KEYWORDS: process analytical technology; capture step; Protein A chromatography; selective antibody quantification; partial least squares regression

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Matthias Rüdert and Nina Brestrich contributed equally to this work.

Correspondence to: J. Hubbuch

Contract grant sponsor: European Union's Horizon 2020 Research and Innovation Programme

Contract grant number: 635557

Received 17 May 2016; Revision received 20 July 2016; Accepted 18 August 2016

Accepted manuscript online 20 August 2016;

Article first published online 21 September 2016 in Wiley Online Library (<http://onlinelibrary.wiley.com/doi/10.1002/bit.26078/abstract>).

DOI 10.1002/bit.26078

Introduction

A capture step is the first unit operation in the protein purification process which is used to bind the target protein from crude harvested cell culture fluid (HCCF). It increases product concentration as well as purity and prevents proteolytic degradation. Due to its high selectivity, Protein A capture is widely used in current monoclonal antibody (mAb) purification platform processes (Hahn et al., 2006; Shukla et al., 2007; Shukla and Thömmes, 2010; Tarrant et al., 2012; Tsukamoto et al., 2014).

A difficulty in Protein A capture is a lack of real-time analytics for mAb quantification in the HCCF and in the column effluent during loading. Since both the mAb and impurities contribute to the absorption at 280 nm (A_{280}), single wavelength measurements are not suitable as selective analytics (Gupta, 2002). To determine the mAb titer in the HCCF, elaborate offline analytics is commonly performed (Fahrner and Blank, 1999b). As mAb titers are influenced by variability in the cell culture, this offline analytics has to be repeated for every lot in order to adapt the load volume onto the column (Fahrner and Blank, 1999a). While this results in a reduced productivity in batch mode, the bottleneck of suitable real-time analytics has to be overcome to enable continuous mAb purification.

In addition to the mAb titer in the HCCF, the optimal load volume onto the column is also influenced by the resin capacity. Due to leaching and degradation of the Protein A ligands as well as pore and ligand blocking by leftover impurities or product, the capacity of the resin decreases over cycle time (Jiang et al., 2009). In batch mode, a conservative loading is commonly applied to avoid breakthrough of the expensive product at the cost of productivity. In contrast to that, columns are overloaded in continuous mode to maximize productivity (Angarita et al., 2015). In this case, the determination of the the percentual product breakthrough is necessary for process control (Warikoo et al., 2012).

To perform (near) real-time process monitoring and control, several process analytical technology (PAT) tools have been developed to enable fast mAb quantification in the cell culture fluid and in the column effluent during loading. For instance, atline mid-IR spectroscopy in combination with multivariate data analysis has been applied for secreted mAb quantification during a Chinese Hamster Ovary (CHO) cell culture process (Capito et al., 2015). Selective mAb quantification in upstream processing was also successfully realized by atline matrix-assisted laser desorption/ionization mass spectrometry (Steinhoff et al., 2016). For the

control of the load phase of a two column continuous Protein A chromatography process, which was connected to a CHO perfusion culture, atline analytical chromatography was applied (Karst et al., 2015). Atline monitoring however, bears the risk of human errors resulting in contamination, time-delays, or missing data.

In order to minimize human impact, automated sampling can be applied. Automated analytical chromatography has been used in upstream processing to monitor the mAb titers (Chase, 1986; Ozturk et al., 1995; Paliwal et al., 1993). In downstream processing, this technique was successfully used for mAb quantification in the column effluent during the load phase of Protein A chromatography. As soon as 1% mAb breakthrough was detected, the load phase was automatically terminated (Fahrner and Blank, 1999a). Automated analytical chromatography is relatively easy to develop and equipment is commercially available. However, the equipment is expensive and the technique error-prone. Besides from the risk of contamination, the time delay between sampling and analytical results bears the risk of late reaction or requires a slow-down of the process.

PAT tools that operate in real-time, such as UV-based methods, overcome these limitations. In a patent application, a UV-based control method for determining binding capacities in Protein A capture was disclosed (Patent WO2010151214A1, also described in Warikoo et al., 2012). The method is based on the calculation of a difference signal between two detectors situated at the column in- and outlet. During the load phase, the post-column signal is supposed to stabilize and is referred to as impurity baseline. As soon as the mAb breaks through, there is an increase in the post-column UV signal above the impurity baseline which corresponds to a breakthrough level of the product. Consequently, the method is very suitable for determining column switching times in continuous Protein A capture. It allows for an equal loading in terms of percentual breakthrough regardless of the mAb titer variability in the feed or decreasing column capacities. However, it requires two detectors posing a risk of unequal detector drifts. A further limitation might be displacement effects of contaminants that prevent a stabilized impurity baseline. The technique might also be limited to the equipment of the future patent holder.

Another recently published UV/Vis-based method for monitoring and control in protein chromatography applies UV/Vis absorption spectra instead of single wavelength measurements (Brestrich et al., 2014, 2015). Different protein species exhibit distinct variations in their UV absorption spectra. Consequently, Partial Least Squares Regression (PLS) technique has been used to correlate absorption spectra with selective protein concentrations. The method was successfully applied for a selective inline protein quantification and for product purity-based pooling decisions in real-time. However, no load control in Protein A chromatography has been performed so far using this technique.

In this study, PLS models correlating UV/Vis absorption spectra with mAb concentrations were applied for real-time monitoring and control of the load phase in Protein A chromatography. In contrast to previous publications in this field, this application requires the monitoring of one protein in the background of many protein and non protein-based contaminants. For the PLS model calibration, several breakthrough experiments were performed and the corresponding absorption spectra of the effluent were acquired. In order to generate variable mixing ratios of mAb and

contaminants for a PLS model training data set, experiments with variable mAb titers in the feed were performed. The column effluent was collected in fractions and analyzed using analytical Protein A chromatography. The recorded absorption spectra were averaged according to the fraction time and correlated with the determined mAb concentrations using PLS technique. The PLS model was eventually applied for a real-time control of the load phase and terminated loading, when 5% or 50% product breakthrough was reached.

Materials and Methods

Cell Culture Fluid and Buffers

HCCF and mock were obtained from Lek Pharmaceuticals d.d. (Mengeš, Slovenia) and stored at -80°C before experimentation. The HCCF and mock were filtered with a cellulose acetate filter with a pore size of 0.22 μm (Pall, Port Washington, NY) before use. In order to achieve a variable mAb concentration in the feed, the HCCF was diluted with mock.

For all preparative runs, the following buffers were applied: Equilibration with 25 mM tris and 0.1 M sodium chloride at pH 7.4, wash with 1 M tris and 0.5 M potassium chloride at pH 7.4, elution with 20 mM citric acid at pH 3.6, sanitization with 50 mM sodium hydroxide and 1 M sodium chloride, and storage with 10 mM sodium phosphate, 130 mM sodium chloride, 20% ethanol.

For analytical Protein A chromatography, column equilibration was carried out using a buffer with 10 mM phosphate (from sodium phosphate and potassium phosphate) with 0.65 M sodium ions (from sodium chloride and potassium chloride) at pH 7.1. Elution was performed with the same buffer, but titrated to pH 2.6 with hydrochloric acid. All buffer components were purchased from VWR, West Chester, USA. The buffers were prepared with Ultrapure Water (PURELAB Ultra, ELGA LabWater, Viola Water Technologies, Saint-Maurice, France), filtrated with a cellulose acetate filter with a pore size of 0.22 μm (Pall), and degassed by sonification.

Chromatographic Instrumentation

All preparative runs were realized with an Akta Pure 25 purification system controlled with Unicorn 6.4.1 (GE Healthcare, Chalfont St Giles, UK). The system was equipped with a sample pump S9, a fraction collector F9-C, a column valve kit (V9-C, for up to 5 columns), a UV-monitor U9-M (2 mm pathlength), a conductivity monitor C9, and an I/O-box E9. Additionally, an UltiMate 3000 diode array detector (DAD) equipped with a semi-preparative flow cell (0.4 mm optical pathlength) and operated with Chromeleon 6.8 (Thermo Fisher Scientific, Waltham, MA) was connected to the Akta Pure. The DAD was positioned between the conductivity monitor and the fraction collector.

The communication between Unicorn and Chromeleon was implemented analogous to the protocol published in (Brestrich et al., 2014). Shortly, Unicorn triggers the DAD data acquisition by sending a digital signal to a Matlab script (MathWorks, Natick, MA), which communicates with Chromeleon via a Visual Basics for Application Macro (Microsoft, Redmond, WA). If a certain condition such as a defined mAb concentration is fulfilled, the

Matlab script sends a signal back to Unicorn to terminate a phase in the chromatographic method.

References analysis of collected fractions was performed using a Dionex UltiMate 3000 rapid separation liquid chromatography system (Thermo Fisher Scientific). The system was composed of a HPG-3400RS pump, a WPS-3000 analytical autosampler, a TCC-3000RS column thermostat, and a DAD3000RS detector.

Chromatography Runs

In order to generate variable mixtures between mAb and impurities for the PLS model calibration and validation, breakthrough experiments with variable mAb titers in the feed were performed. The mAb titers in the different experiments were 2.7, 2.85, 3, 3.15, and 3.3 mg/mL. For each experiment, a Sartobind 2 mL Protein A membrane (Sartorius, Göttingen, Germany) was first equilibrated for three membrane volumes (MVs) and then loaded with 33.15 mg mAb. At the beginning of the load phase, the DAD was triggered to record absorption spectra between 200 and 410 nm and the membrane flow-through was collected in 200 mL fractions. After a first wash with equilibration buffer for 4.5 MVs, the membrane was flushed with wash buffer for 5.5 MVs and with equilibration buffer for 4.5 MVs. Elution was carried out for 5 MVs followed by a re-equilibration of 1.5 MVs. Eventually, the column was sanitized for 5 MVs, and between the runs, kept in the storage buffer. The flow rate was 1 mL/min for all phases and experiments.

Analytical Chromatography

As displayed in Figure 1, the collected fractions of all runs were examined by analytical Protein A chromatography to obtain the mAb concentrations. For each sample, a 2.1×30 mm POROS prepacked Protein A column (Thermo Fisher Scientific, Foster City, CA) was equilibrated with 2.6 column volumes (CVs) of equilibration buffer, flowed by an injection of 20 mL sample. The column was then equilibrated with 0.8 CVs of equilibration buffer and eluted with 1.4 CVs of elution buffer. The flow rate was 2 mL/min for all phases and experiments.

Data Analysis

For the correlation of the absorption spectra with the mAb concentrations, PLS technique was applied using SIMCA (MKS Data Analytics Solutions, Umeå, Sweden). SIMCA applies the NIPALS-algorithm for PLS. Before performing PLS, all spectra were preprocessed by mean centering using SIMCA. PLS finds variation in the spectral data matrix, which is relevant for the correlation with the mAb concentrations, and thereby, separates information in the matrix from detector noise (Eriksson et al., 2006; Höskuldsson, 1988; Martens and Næs, 1989). In order to achieve this separation, collinearity in the data are reduced by summarizing variables (here wavelengths) with similar information in *latent variables* (LVs). This is done in a way such that the content of relevant information for the correlation included in each LV is highest for the first LV and decreases for the following ones. The number of applied LVs in a PLS model is hence a measure of data reduction and only a few LVs are required to obtain the correlation between absorption spectra and mAb concentrations.

The number of applied LVs has to be evaluated thoroughly to avoid under- or overfitting of a model. In order to determine a reasonable number of LVs, the root mean square error (RMSE) for the prediction of validation samples is usually determined in dependence on the number of LVs applied in a PLS model. The minimum corresponds to the optimal number of LVs. In this study, cross validation was performed to determine an optimal number of LVs. Therefore, the calibration data were separated into seven groups. One group was then excluded during model calibration and the RMSE for these samples was calculated subsequently. For every number of LVs, this procedure was performed until each group was excluded. Based on the so obtained number of LVs, completely independent runs were predicted to evaluate the final models.

A first PLS model calibration was based on the results of the runs with the following mAb titers in the feed: 2.7, 2.85, 3.15, and 3.3 mg/mL. The results of the corresponding spectral acquisitions are time, wavelength, and absorption 3D-fields. The 3D-fields were averaged in time according to the fraction duration as displayed in Figure 1. The results of these calculations were stored in an absorption matrix. Afterward, PLS was carried out to correlate the mAb concentrations of the collected fractions with the corresponding absorption matrix.

For lower protein concentrations, a second PLS model was calibrated. Only samples with mAb concentrations below 0.5 mg/mL were considered in the model calibration. For those samples, a background subtraction was performed. As soon as the change in absorption signal after impurity breakthrough fell under a predefined threshold, an average absorption was calculated for every wavelength. This impurity background was subtracted from the absorption of all following data points.

Real-Time Monitoring and Control

The first calibrated PLS model was subsequently applied for a real-time monitoring of the mAb concentrations in a run with a mAb titer of 3 mg/mL in the feed. While the calibration of the PLS model was performed using averaged spectra, predictions were based on the 3D-fields. This means that the a spectrum at each time point was applied to predict the mAb concentrations. The absorption spectra of the effluent were recorded and translated into mAb concentrations in real-time by the calibrated PLS model. The calculation of the mAb concentrations was executed in Matlab. In a first run, a stop criterion of 1.5 mg/mL mAb concentration (50% product breakthrough) was set in the Matlab evaluation script. As soon as the termination criterion was reached, a digital signal was sent from Matlab to Unicorn and the load phase was terminated. In a second run, the stop criterion to terminate the load phase was set to a target concentration of 0.15 mg/mL (5% product breakthrough). For this condition, the second PLS model was used.

Results and Discussion

The setup described in the Section Material and Methods was used to monitor the breakthrough of mAb with UV/Vis spectroscopy in combination with PLS modeling. To calibrate the PLS model, four chromatographic runs at mAb concentrations of 2.7, 2.85, 3.15, and 3.3 mg/mL in the feed were performed and analyzed by offline

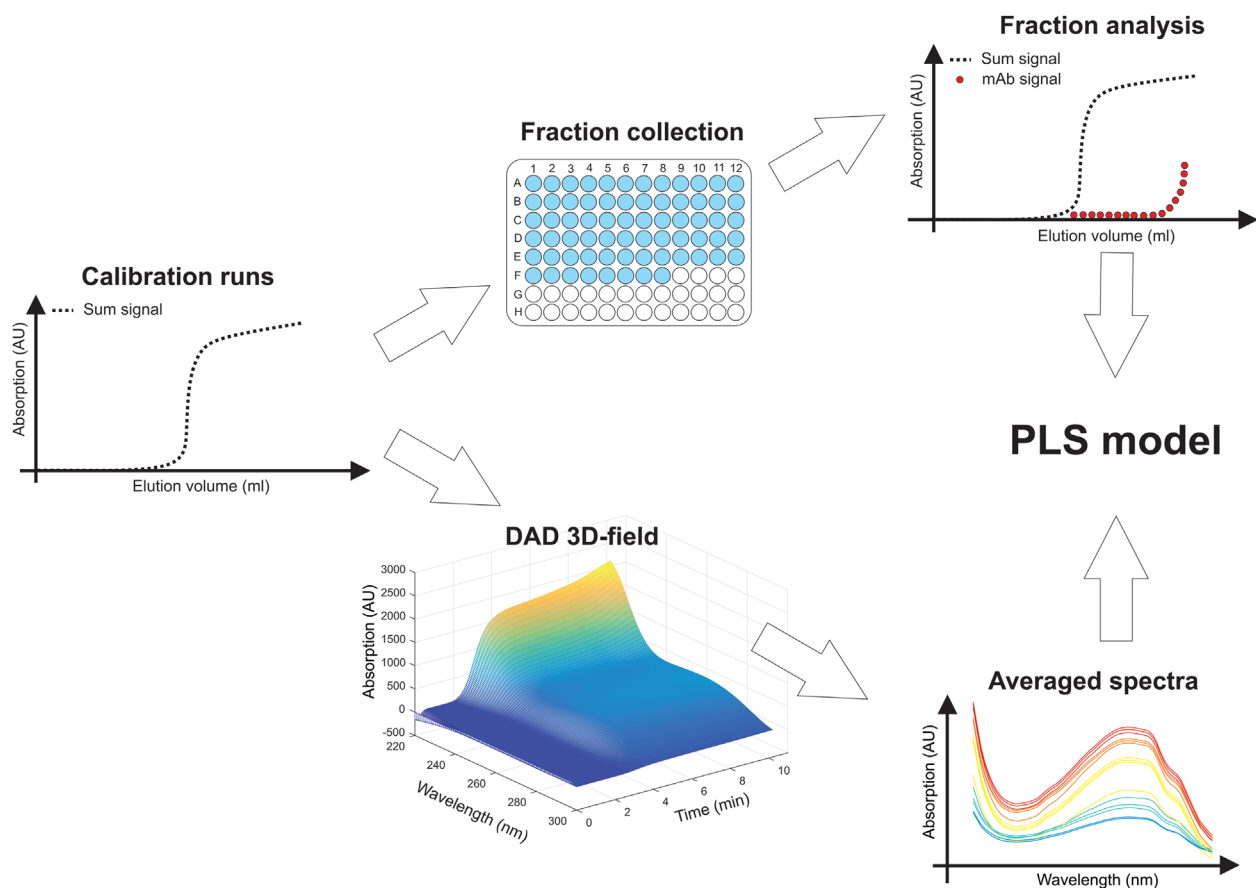


Figure 1. Experimental procedure for the PLS model calibration: For each calibration run, 200 mL fractions were collected and analyzed by analytical Protein A chromatography to obtain the mAb breakthrough curves. In addition, averaged spectra corresponding to the fraction size were calculated from the time, wavelength, and absorption 3D-field. Averaged spectra and mAb concentrations were eventually correlated using PLS technique.

analytics. The model was eventually confirmed by performing a real-time control of two runs with a mAb titer of 3 mg/mL. The difference in the mAb titers in the feed ensured variable mixing ratios between product and contaminants. This was done to imitate variability in upstream processing and to span a calibrated design space for the PLS model.

PLS Model Calibration

The results of the model calibration are illustrated by Figure 2. It compares the A_{280} (recorded at a pathlength of 0.4 mm and displayed as dashed black line) to the concentrations measured by offline analytics (blue bars) and the signal calculated by the calibrated PLS model (solid red lines). The number of LVs was set to four based on a minimal RMSE of 0.08 mg/mL in the cross validation. The calibrated PLS model was applied to evaluate all 3D-fields. In contrast to model calibration, where averaged spectra were used, the spectral raw data at each time point was translated into concentrations. The estimated concentrations by the PLS model closely follow the measured values by offline analytics. It is worth noting that no clear plateau of the A_{280} is reached after the breakthrough of media components. Instead, the A_{280} continuous to

increase. This may be caused by different impurities being retained differently on the membrane. Indeed, it has previously been shown, that major interactions between HCPs, the stationary phase, and mAbs may occur (Aboulaich et al., 2014; Shukla and Hinckley, 2008). The advent of mAb breakthrough cannot be clearly distinguished from A_{280} alone. Based on the multivariate spectral data, the PLS model is able to predict protein concentrations, which allows for real-time monitoring and control.

Real-Time Monitoring and Control

For the confirmation of the obtained results, the calibrated PLS model was used to control the load phase of a Protein A capture step in real-time. In a first run, a target breakthrough concentration of 1.5 mg/mL was set, which corresponds to 50% product breakthrough. Figure 3A shows the A_{280} (dashed black line), the real-time prediction of mAb concentrations (solid red line) and the corresponding offline analytics (blue bars). The model reached an RMSE for prediction of 0.06 mg/mL compared to the offline analytics. This approach may be of interest for controlling a continuous chromatography system. In this context, the prediction of lower mAb concentrations is not so crucial.

For a possible application in batch chromatography, the sensitivity of the model was further improved. A second PLS model was hence

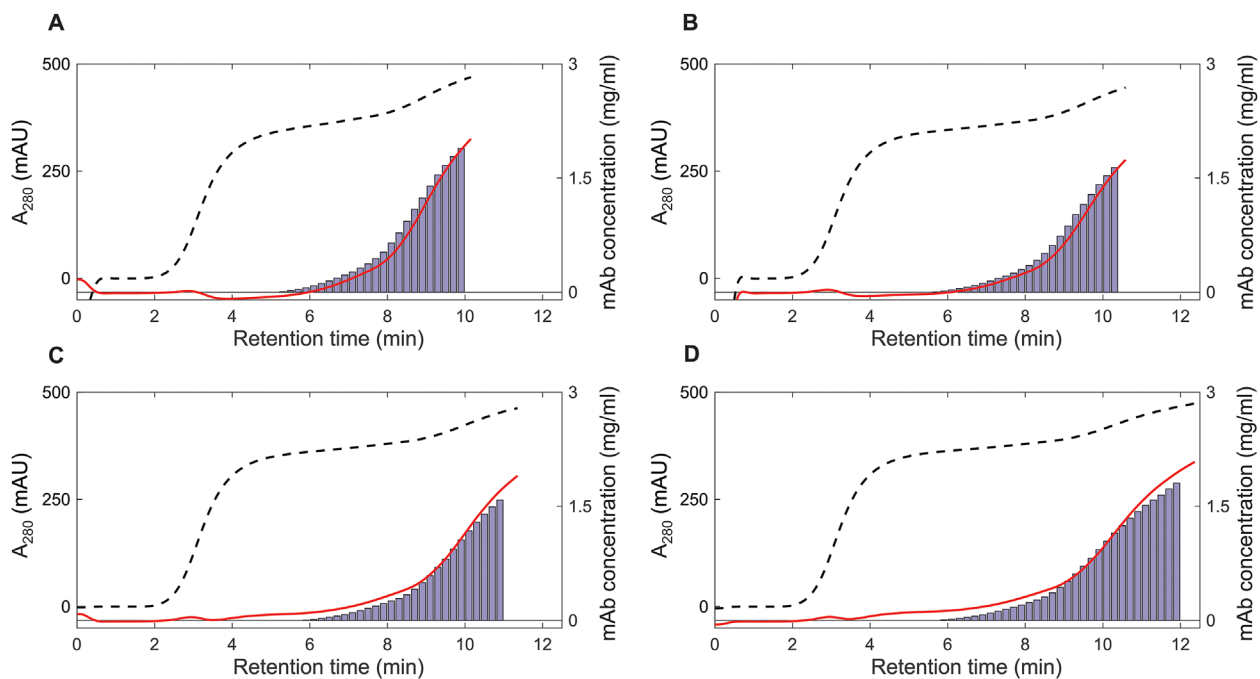


Figure 2. Results of the PLS model calibration. The A_{280} (measured at a pathlength of 0.4 mm and displayed as dashed black line) is compared with the results of the offline analytics for mAb quantification (blue bars). The PLS model prediction is illustrated as red lines. The four runs exhibited variable mAb titers in the feed **A:** 3.3 mg/mL, **B:** 3.15 mg/mL, **C:** 2.85 mg/mL, **D:** 2.7 mg/mL.

calibrated based on the calibration data set as described in the method section. The recalibration was performed to increase the sensitivity in the given concentration range. It was noticed, that it is difficult to accurately calibrate a PLS model for broad concentration ranges. By reducing the concentration calibration range, smaller RMSE values could be achieved. The model was used predict and stop a load phase in a second run at 0.15 mg/mL, which corresponds to 5% product breakthrough. The results of this second run are displayed in Figure 3B. As an impurity background was subtracted to increase the

sensitivity of the method, the A_{280} suddenly decreases. The second PLS model reached an RMSE for prediction of 0.01 mg/mL.

During both runs, the respective load phases were successfully terminated close to the intended breakpoints. In Table I, a summary of intended and measured mAb concentrations in the last fraction of both confirmation runs is shown. The Matlab script sent a digital signal to Unicorn and terminated the load phase, when the targeted breakthrough concentration was reached. As the targeted breakthrough set points were concentrations at discrete time points, they are expected

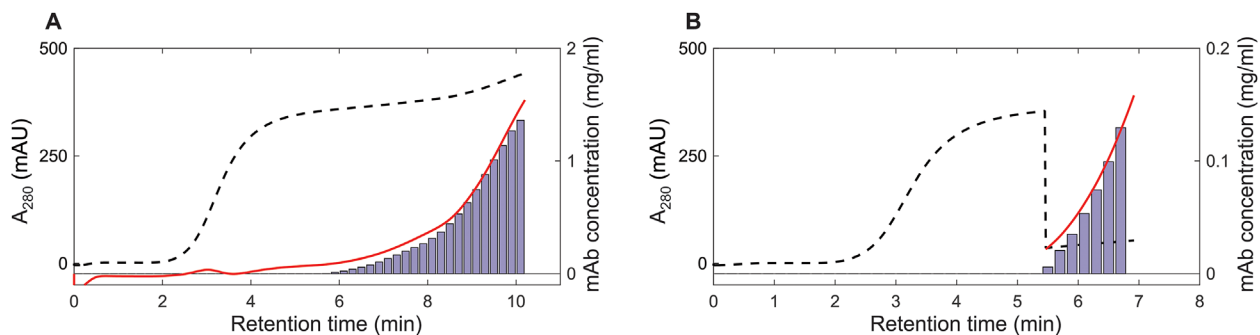


Figure 3. Results of the model evaluation by performing a real-time control of the load phase using a mAb titer of 3 mg/mL in the feed. The PLS model prediction (red lines) is compared with the results of the offline analytics (blue bars) as well as the A_{280} (measured at a pathlength of 0.4 mm and displayed as dashed black line). The load phase was automatically terminated, when a mAb concentration in the effluent of **A:** 1.5 mg/mL or **B:** 0.15 mg/mL was reached. The sudden decrease in the A_{280} arises from the background subtraction.

Table I. Results of both confirmation runs: The targeted concentration to terminate loading is compared with the mAb concentration in the last fraction as determined by offline analytics. PLS model prediction for the last fraction based on an averaged absorption spectrum is shown for comparison.

c_{target} [mg/mL]	$c_{\text{analytics}}$ [mg/mL]	$c_{\text{mean, PLS}}$ [mg/mL]
1.50	1.36	1.47
0.150	0.129	0.126

to be slightly higher than the concentrations of the last fraction determined by offline analytics. This was observed for both confirmation runs (cf. Table I). For an easier comparison between model and offline analytics, a concentration based on an averaged absorption spectrum was calculated for the last fractions of both runs and compared with the corresponding offline analytics. For the first run, the deviation between prediction and reference was 8.0%, while for the second run a deviation of 2.3% was found. This demonstrates that the described method can be successfully used to control the load phase in a Protein A capture step.

Conclusion and Outlook

A real-time monitoring and control of the load phase in a Protein A capture step was successfully realized in this study. It was demonstrated that PLS modeling on UV/Vis absorption spectra can be applied to quantify mAb in the effluent during the load phase despite of the background of many protein and non protein-based impurities. Based on the quantification, the load phase was automatically terminated, when a product breakthrough concentration of 1.5 mg/mL or 0.15 mg/mL was reached. Consequently, the proposed method has potential for the monitoring and control of capture steps at large scale production. In batch chromatography, the loading volume may be defined dynamically to allow for increased resin capacity utilization while still keeping the product loss small. Additionally, time-consuming offline determination of the mAb titer in HCCF could be eliminated. The method may also be interesting for controlling column switching times in continuous chromatographic capture steps. Future challenges are especially related to the scale up and robustness of the method. Regarding the latter, especially upstream variations should be calibrated into the PLS model. Research will now focus on the migration of the method to the control of continuous capture steps.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 635557. We kindly thank Lek Pharmaceuticals d.d. (Mengeš, Slovenia) for providing the HCCF and mock. This article is related to a patent application filed at the European Patent Office under the number EP15196599.3.

References

Aboulaich N, Chung WK, Thompson JH, Larkin C, Robbins D, Zhu M. 2014. A novel approach to monitor clearance of host cell proteins associated with monoclonal anti-bodies. *Biotechnol Prog* 30:1114–1124.

- Angarita M, Müller-Spätth T, Baur D, Lievrouw R, Lissens G, Morbidelli M. 2015. Twin-column capture SMB: A novel cyclic process for protein A affinity chromatography. *J Chromatogr A* 1389:85–95.
- Brestrich N, Briskot T, Osbergerhaus A, Hubbuch J. 2014. A tool for selective inline quantification of co-eluting proteins in chromatography using spectral analysis and partial least squares regression. *Biotechnol Bioeng* 111:1365–1373.
- Brestrich N, Sanden A, Kraft A, McCann K, Bertolini J, Hubbuch J. 2015. Advances in inline quantification of co-eluting proteins in chromatography: Process-data-based model calibration and application towards real-life separation issues. *Biotechnol Bioeng* 112:1406–1416.
- Capito F, Zimmer A, Skudas R. 2015. Mid-infrared spectroscopy-based analysis of mammalian cell culture parameters. *Biotechnol Prog* 31:578–584.
- Chase HA. 1986. Rapid chromatographic monitoring of bioprocesses. *Biosensors* 2:269–286.
- Eriksson L, Johansson E, Kettaneh-Wold N, Trygg J, Wikström C, Wold S. 2006. Multi-and megavariable data analysis. Umeå: Umetrics Academy. p 95.
- Fahrner RL, Blank GS. 1999a. Real-time control of antibody loading during protein A affinity chromatography using an on-line assay. *J Chromatogr A* 849:191–196.
- Fahrner RL, Blank GS. 1999b. Real-time monitoring of recombinant antibody breakthrough during protein A affinity chromatography. *Biotechnol Appl Biochem* 29:109–112.
- Gupta M. editor. 2002. *Methods for affinity-based separations of enzymes and proteins*. Basel, Switzerland: Birkhäuser Basel.
- Hahn R, Shimahara K, Steindl F, Jungbauer A. 2006. Comparison of protein A affinity sorbents III. Life time study. *J Chromatogr A* 1102:224–231.
- Höskuldsson A. 1988. PLS regression methods. *J Chemom* 2:211–228.
- Jiang C, Liu J, Rubacha M, Shukla AA. 2009. A mechanistic study of protein A chromatography resin lifetime. *J Chromatogr A* 1216:5849–5855.
- Karst D, Steinebach F, Morbidelli M. 2015. Integrated continuous processing for the manufacture of monoclonal antibodies, presented at Integrated Continuous Biomanufacturing II, Berkeley CA, USA.
- Martens H, Næs T. 1989. *Multivariate calibration*. Chichester, UK: John Wiley & Sons.
- Ozturk SS, Thrift JC, Blackie JD, Naveh D. 1995. Real-time monitoring of protein secretion in mammalian cell fermentation: Measurement of monoclonal antibodies using a computer-controlled hplc system (biocad/rpm). *Biotechnol Bioeng* 48:201–206.
- Paliwal S, Nadler T, Wang D, Regnier F. 1993. Automated process monitoring of monoclonal antibody production. *Anal Chem* 65:3363–3367.
- Shukla AA, Hinckley P. 2008. Host cell protein clearance during protein A chromatography: Development of an improved column wash step. *Biotechnol Prog* 24:1115–1121.
- Shukla AA, Hubbard B, Tressel T, Guhan S, Low D. 2007. Downstream processing of monoclonal antibodies—Application of platform approaches. *J Chromatogr B* 848:28–39.
- Shukla AA, Thömmes J. 2010. Recent advances in large-scale production of monoclonal antibodies and related proteins. *Trends Biotechnol* 28:253–261.
- Steinhoff RF, Karst DJ, Steinebach F, Kopp MR, Schmidt GW, Stettler A, Krismer J, Soos M, Pabst M, Hierlemann A, Morbidelli M, Zenobi R. 2016. Microarray-based MALDI-TOF mass spectrometry enables monitoring of monoclonal antibody production in batch and perfusion cell cultures. *Methods* 104:33–40.
- Tarrant RDR, Velez-Suberbie ML, Tait AS, Smales CM, Bracewell DG. 2012. Host cell protein adsorption characteristics during protein A chromatography. *Biotechnol Prog* 28:1037–1044.
- Tsukamoto M, Watanabe H, Ooishi A, Honda S. 2014. Engineered protein A ligands, derived from a histidine-scanning library, facilitate the affinity purification of IgG under mild acidic conditions. *J Biol Eng* 8:1–9.
- Warikoo V, Godawat R, Brower K, Jain S, Cummings D, Simons E, Johnson T, Walther J, Yu M, Wright B, McLarty J, Karey KP, Hwang C, Zhou W, Riske F, Konstantinov K. 2012. Integrated continuous production of recombinant therapeutic proteins. *Biotechnol Bioeng* 109:3018–3029.