# Modeling & Simulation of Affinity Chromatography & Investigation of CaptureSMB

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Master Thesis 2018

LUND UNIVERSITY

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June 2018

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Picture on front page: HiTrap MabSelect Sure column and HiTrap MabSelect Prisma A column Photo by Dennis Bogren

# Preface

This report is the result of my Master's Thesis that has been carried out at the Department of Chemical Engineering at the Faculty of Engineering, Lund University, Sweden from January to June 2018.

I would like to thank and express my gratitude to Professor Bernt Nilsson for giving me the opportunity to perform this Master Thesis at the department of Chemical Engineering, Lund University. I would also like to thank him for his advice and sharing his great knowledge.

Next in line I would like to say thanks to my supervisor Associate Senior Lecturer Niklas Andersson for your support and guiding me through this project. I would also like to say thanks to PhD Anton Sellberg for the introduction in different modelling, to PhD Joaquin Gomis Fons, PhD Anton Löfgren and Simon Tallvod for all the help in the laboratory and with orbit.

Last but not least I would like to thank the rest of the Master's Thesis students for Professor Bernt Nilsson and the rest of the staff at the department of Chemical Engineering, Lund University for the enjoyable atmosphere and pleasant discussions.

# Abstract

Chromatography was first described in the 1903 by a Russian scientist. Later on chromatography was almost forgotten until the 1940s when two British scientist published a study about liquid chromatography. They were later awarded with the Nobel prize in chemistry for their work in chromatography. Chromatography has since then developed a lot and nowadays several different kinds of liquid chromatography are used. Liquid chromatography are foremost used in the biochemistry, biotechnology, medicine and the pharmaceutical industry.

This study had focused on affinity chromatography and to be able to simulate affinity chromatography. Affinity chromatography is when a ligand matrix are used as the stationary phase in the columns. This ligand matrix only binds in to a specific protein in this case IgG and lets the rest of the substances pass by. In this way IgG is separated from the other proteins and contaminations. The IgG is then eluted from the column by a change in the pH value.

The work was started with some experimental runs and then a model was combined to fit the experimental data. When the model got a moderately fit to the experimental data it was calibrated to get a better fit by the function lsqcurvefit in MATLAB. With the calibration the exact parameters were given for the model.

The next part of the study was to investigate the capacity of the protein A columns HiTrap MabSelect Sure and HiTrap MabSelect Prisma A. The capacity of the columns was investigated by some breakthrough experiments and at the same time the breakthrough profiles were investigated. It was seen that HiTrap MabSelect Prisma A had a greater capacity of about 45mg IgG/ml resin than HiTrap MabSelect SuRe. It was also found that with a lower flowrate, a higher slope was achieved for both the columns.

The last part in this study was the investigation of CaptureSMB. CaptureSMB is a combination of affinity chromatography and simulated moving bed (SMB). It uses two columns that by shifting valves gives the appearance that the columns shifts places. CaptureSMB has four different steps. The first step is to load the first column and when breakthrough occurs it is loaded on to the second column. The second step is to elute, strip, cip and regenerate the first column while the second column gets filled. The third step is when the first column are regenerated it is placed after the second column to capture when breakthrough occurs on the second column. The fourth and last step is when the second column are eluted, striped, ciped and regenerated while the first column are loaded. The proposed process for affinity chromatography in this study used four versatile valves, two UV sensors, one conductivity sensor, one pH sensor, three pumps, three inlet valves, one injection valve and two columns.

Keywords: Affinity chromatography, simulation of chromatography, breakthrough curve, CaptureSMB

# Sammanfattning

Kromatografi var först beskrivet av en rysk vetenskapsman 1903. Efter det var kromatografi stort sett bortglömt tills två brittiska vetenskapsmän på 1940-talet publicerade en studie om flytande kromatografi. De blev senare belönade med nobelpriset i kemi 1952 för deras arbete inom kromatografi. Kromatografi har sedan dess utvecklats mycket och i dag finns flera olika sorters kromatografi. Flytande kromatografi används mestadels inom biokemi, bioteknologi, medicin och läkemedelsindustrin.

Denna studie har framförallt fokuserat på affinitetskromatografi. Mestadels på att simulera affinitetskromatografi och sedan försöka ändra parametrar så att simuleringarna passar den experimentella data som fåtts. Vid affinitetskromatografi så har man bundit in en ligandmatris i den stationära fasen på kolonen. Denna ligandmatris låter endast ett protein binda in till den vilket i detta fallet är IgG. Resterande ämnen i lösningen som renas upp åker bara rakt genom kolonnen. För att sedan eluera ut proteinet ur kolonnen så sänks pH-värdet.

Till en början så gjordes experiment för att då fram lite användbar data. Därefter så sattes en modell samman för att försöka passas till den experimentella data som fåtts. När den experimentella data och modellen passade någorlunda bra så gjordes en kalibrering i MATLAB på modellen med hjälp av funktionen lsqcurvefit. Med denna funktion ficks de parametrar till modellen som gav en så bra passning som möjligt.

Nästa del av studien var att undersöka kapaciteten av IgG i två olika affinitets kolonner. Dessa kolonner var av sorterna HiTrap MabSelect Sure och HiTrap MabSelect Prisma A. Kapaciteten undersöktes genom genombrottsexperiment och samtidigt undersöktes även genombrottskurvan för de båda kolonnerna. Det syntes att HiTrap MabSelect Prisma A hade nästan 45mg IgG/ml stationärfas högre kapacitet än HiTrap MabSelect SuRe. Det kunde även utläsas att vid en lägre flödeshastighet genom kolonen så blev genombrottskurvan brantare för båda kolonnerna.

Den sista delen av denna studien var undersökningen av CaptureSMB. CaptureSMB är en kombination av affinitets kromatografi och Simulated Moving Bed. Processen använder två kolonner och genom omslag av ventiler ger upphov till att det ser ut som att kolonnerna byter plats. CaptureSMB har fyra olika steg. Det första steget är att ladda på första kolonnen och när genombrottet sker så fångas det upp av den andra kolonnen. Det andra steget är när man eluerar, rengör och regenererar den första kolonen samtidigt som den andra kolonnen laddas på. Steg tre blir sen när den första kolonnen placeras efter den andra kolonen och fångar upp det som läcker genom vid genombrottet av den. Steg fyra blir sen när den andra kolonen elueras, rengörs och regenereras samtidigt som första kolonen laddas på igen. Den uppställning som föreslås i denna rapport för CaptureSMB använder fyra versatile ventiler, två UV sensorer, en konduktivitetsmätare, en pH mätare, tre pumpar, tre buffert ventiler, en injektions ventil och två kolonner.

Nyckelord: Affinitetskromatografi, simulering av kromatografi, genombrottskurva, CaptureSMB

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# 1 Introduction

Chromatography is a technique used for purification and separation of biological and chemical substances. Chromatography can be used both in laboratory scale and in industrial process scale (Kaiser & Dybowski, 2000). Chromatography is a two-phase system, one mobile phase and one stationary phase. It is the difference in the equilibrium constant for the components placed in a two-phase system that makes the separation (Guiochon, 2012).

Affinity chromatography is a technique involving a bioselective stationary phase. Because of its selective interactions affinity chromatography is more selective than other liquid chromatography methods (Kaiser & Dybowski, 2000). The stationary phase contains of ligands immobilized to an Agarose, cross-linked dextran's or cellulose gel. The target protein that should be purified, adsorbs in to the ligand under favorable conditions for the analyte-ligand complex. This is then eluted by a buffer with conditions that is favorable for the dissociation of the analyte-ligand complex (Hage, 2014).

## 1.1 Aim

The aim of this study was to investigate affinity chromatography. The investigation was divided into different categories. The first category was to develop a simulation model and fit it to experimental data. The simulation was carried out in MATLAB. Experiments were executed and then a simulation was fitted to these experiments. The second category was to look at the breakthrough curve when a column was loaded. The things that were changed during this part were the flowrate and the column type. The third and last category that was studied was CaptureSMB and was also tested in the lab.

# 2 Theory

## 2.1 History of Chromatography

Chromatography was first described in 1903 by M.S Twsett. This when he separated different kinds of chlorophyll and other phytopigments. After this the method was partly forgotten, until the British's Archer Martin and Richard Synge in the 1940 published an underlying study of liquid chromatography (Schmidt-Taub, 2015). In 1952 they were awarded with the Nobel prize in chemistry for their work in chromatography (Nobelprize.org, u.d.). The liquid chromatography has developed a lot since the 1970 and is important within the biochemistry, biotechnology, medicine and the pharmaceutical industry (Kaiser & Dybowski, 2000).

## 2.2 General Chromatography

The basics in chromatography is that there are two phases. One stationary phase that is stagnant and one mobile phase that is flowing through the column (Guiochon, 2012). Usually the liquid chromatography is operated at reversed phase, which is when a water based liquid mobile phase is used and a nonpolar stationary phase. It is also possible to use normal phase chromatography where the mobile phase is an organic solvent and the stationary phase is based of silica or alumina (Jandera & Henze, 2012).

Separation by liquid chromatography can be made at several different ways. Some of the methods are gel filtration, ion exchange chromatography and affinity chromatography. Gel filtration uses the size of the molecules to separate the different components. This is executed by the stationary phases pores, which lets the smaller molecules go in to the pores and therefore gives them a longer residence time than the bigger molecules (Jandera & Henze, 2012). In ion exchange chromatography negative (cation exchangers) or positive (anion exchangers) ions are attached to the stationary phase. These ions attract the ions of the opposite charge from the mobile phase. This is then eluted by a salt mixture which replaces the ions from the mobile phase at the sites on the stationary phase. Depending on the charge of the adsorbed substance they attach differently hard to the stationary phase and therefor also elute at different salt concentrations (Jandera & Lembke, 2012). Affinity chromatography uses a bio specific binding to the adsorption medium (stationary phase) (Cabrera, et al., 2012) and has been used in this study and will be described more thoroughly in chapter 2.3.

## 2.3 Affinity Chromatography

The first time affinity chromatography was successfully used was in 1968 to purify enzymes. Since then innumerable proteins for affinity chromatography has been isolated. One advantages with this is that the protein retain their biological activity after the separation (Lembke, 2012).

The affinity chromatography is based on the same biospecific interactions that occurs in natural biological processes. Most of the time an affinity matrix containing ligands that has been produced in the laboratory. This matrix is specifically produced to purify only the desired protein. Then is the matrix filled in a column and works as a stationary phase. The mobile phase containing the target protein and other components are passed through the column and the protein is adsorbed to the ligand. The properties of the mobile phase is then changed to desorb the protein from the ligand and the protein is isolated and elutes (Lembke, 2012). A schematic picture of this are shown in figure 2.1. The picture has been drawn by the writer with inspiration from (Schmidt-Taub, 2015).

When proteins are purified the impurities will leak through the column, which results in an indication on the UV sensor. This indication will be constant as long as the impurities are constant and this will form a new offset baseline. From this new baseline the UV signal will increase during breakthrough. This baseline has to be neglected when column capacity are calculated (Carta & Jungbauer, 2010).
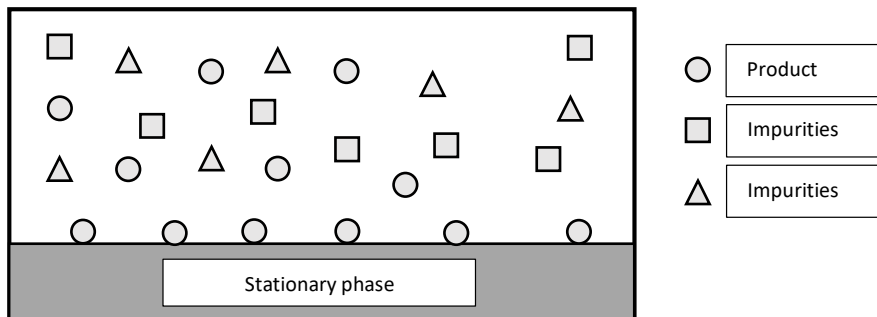


*Figure 2.1. Schematic picture of affinity chromatography drawn by the writer with inspiration from (Schmidt-Taub, 2015).*

## 2.4   CaptureSMB

Simulated moving bed (SMB) was first developed in the 50s. The SMB works in the way that with help of valves that shifts, the columns shifts places which gives a counter current movement of the stationary phase. At the beginning it was used with 3-4 columns for difficult separations where the compounds had a selectivity close to one. The combinations of affinity chromatography and SMB gave rise to CaptureSMB. By using the second column as capture during the first column is loaded, the amount that breakthrough is also recovered. This means that the first column can be loaded to a higher grade than before since no product gets lost. This leads to improvements compared to batch chromatography on the productivity, the amount loaded to the column and the amount of buffer used (Angarita, et al., 2015).

CaptureSMB is a cyclic process, one cycle is complete when all the columns have gone through all the steps in the cycle. A cycle starts with loading to the first column and when it gets saturated and breakthrough occurs it is captured by the second column. When the first column is filled to a desired level the feed flow is stopped. Then buffer is pumped through the first column to wash out the rest of the raw extract in to the second column. When this is done the load to the second column starts and the first column will be eluted, cleaned in place(CIP), striped and regenerated. This because the first column will then be the one that captures at breakthrough. A schematic picture of this can be seen in figure 2.2 The method used for elution, CIP, strip and regeneration was found in (Healthcare, 2017) and can also be seen in Appendix A in table A.1 and the buffers were produced accordingly to table A.2 where buffer A is also called PBS which can be found in (Corporation, u.d.).
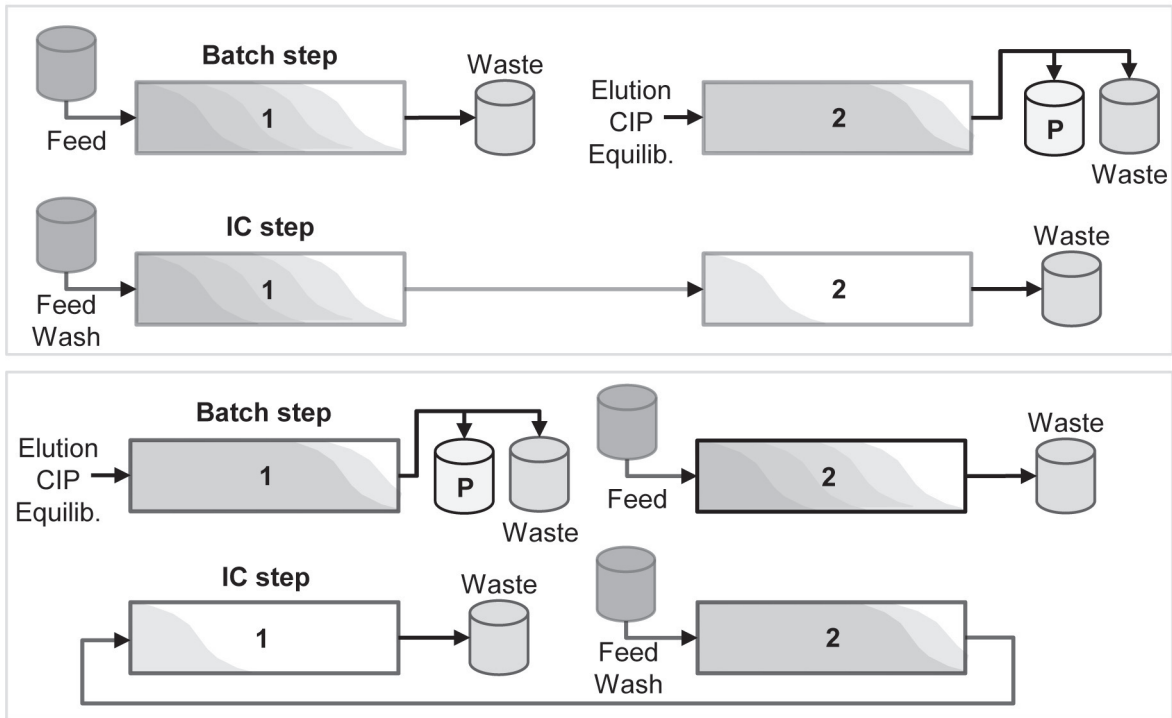
*Figure 2.2. Shows the principle for a complete cycle of CaptureSMB (Angarita, et al., 2015).*

## 2.5 Absorbance to Concentration

To be able to compare the simulations and the experimental result a correlation between absorbance and concentration needs to be found. The correlation that has been used in this study is Beer-lambert law that can be seen in equation 2.1 (Scientific, 2013). The wavelength used for the detection was 280nm which was the only possible wavelength for the UV sensor (Bio-Sciences, 2017). The l in equation 2.1 is the length of the absorbance cell and is defined to 0,2 cm according to (Bio-Sciences, 2017). The c in equation 2.1 is concentration, the ε is the molar absorptivity and the Abs is the absorbance and is the same for equation 3.1.

$$Abs = \varepsilon_{Abs}\, c\, l \qquad\qquad\qquad (2.1)$$

## 2.6 IgG

Immunoglobulin G (IgG) is a sort of monoclonal antibody (mAb) (Bio-Sciences, 2007) which is a part of the human immune system (Mallery, et al., 2010). To purify the mAbs affinity chromatography with specially made columns are used (Bio-Sciences, 2007). The IgG used in experimental setups needs to be stored in a freezer with a temperature below -20°C (Aldrich, 2018). One way to optimize and develop the affinity chromatography process further is to develop new and more effective resins to the columns (BioProcess, 2017).

## 2.7 Breakthrough

Breakthrough occurs when all the adsorption sites are occupied. This leads to that the protein leaking through the column. The amount adsorbed to the column is the area of section A and the amount leaked through is the area of section B in figure 2.3. With the breakthrough experiment it is also possible to calculate the column capacity. This is calculated by approximating a straight vertical line along the breakthrough curve. With this line two triangles are formed, one in the upper right corner of section A and the other one in the bottom left corner

of section B. When these two triangles has the same area the column capacity is where the line and breakthrough curve intersects. A schematic picture of a breakthrough curve can be seen in figure 2.3 (Carta & Jungbauer, 2010).
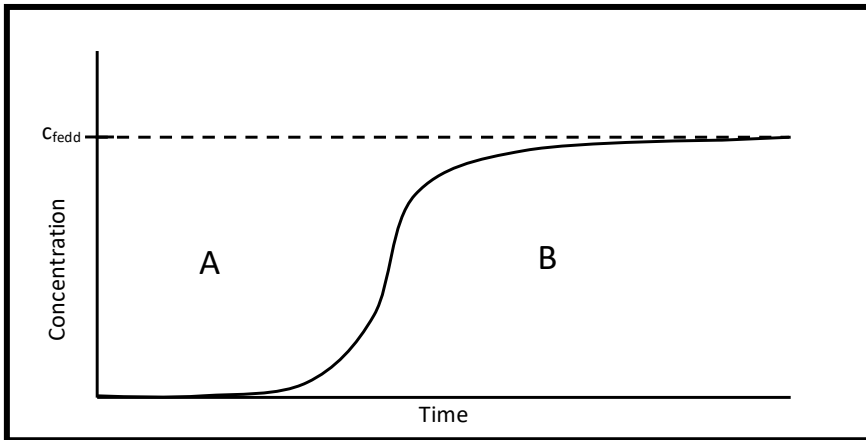


*Figure 2.3. Schematic picture of a breakthrough curve drawn by the writer with inspiration from (Carta & Jungbauer, 2010).*

# 3  Material and Method

## 3.1  Raw Material

In this study a raw extract from the Royal Institute of Technology, Stockholm has been used. In the raw extract IgG was the desired protein and HiTrap MabSelect SuRe 1ml column and HiTrap MabSelect Prisma A 1ml column from Ge Healthcare in Uppsala Sweden has been used to extract the IgG. In CaptureSMB two columns were used and shifts place to be able to run continuously. The columns were attached and run by a ÄKTA pure machine from GE Healthcare, shown in figure 3.1. The ÄKTA pure machine shifts valves to change flow directions and thereby give rise to SMB.
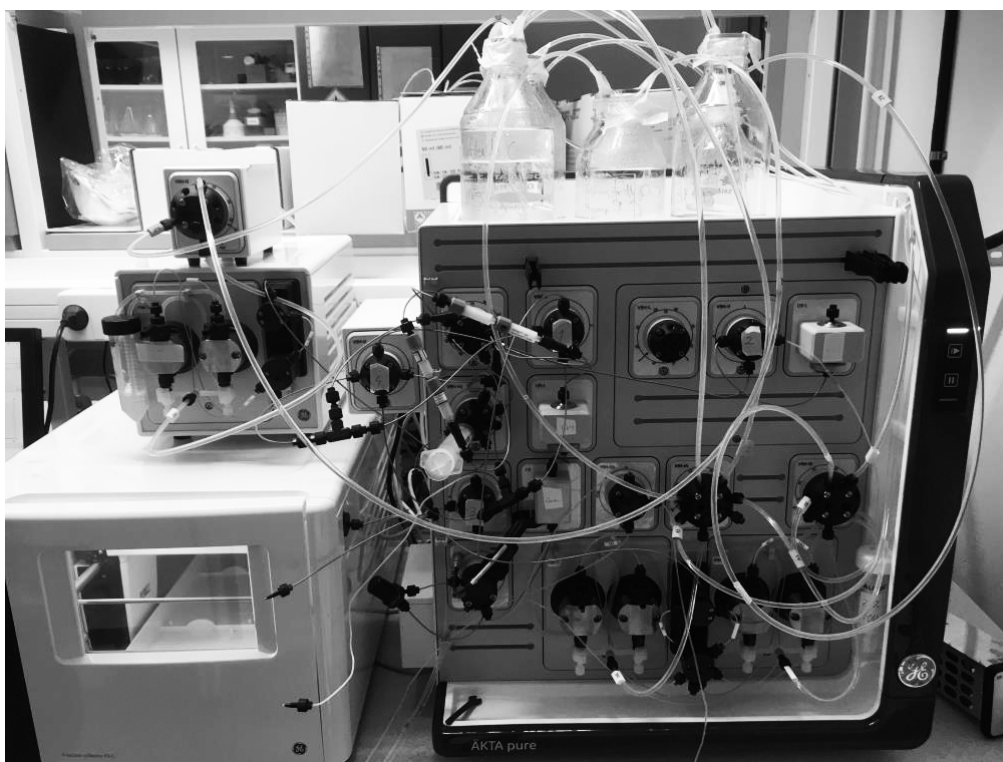


*Figure 3.1. ÄKTA pure machine used in this study.*

## 3.2  Connection and Operation of the ÄKTA Pure Machine

The standard method to control the ÄKTA pure machine is by using the software Unicorn from GE Healthcare. Unicorn is a wide library with methods to operate the machine. The downside with unicorn is the lack of possibility to real-time control, complex scripting and running multiple connected ÄKTA system at the same time. Since this study needed real-time control an alternative method to control the machine was used. The method used was to connect by an OPC connection. OPC stands for "OLE process control" where OLE stands for "Object Linking and Embedding".

To connect to the machine by an OPC a scripting language needs to be used. During this study Python has been used as the scripting language. To improve the usage of the OPC connection has the Department of Chemical engineering at Lunds University created a library with methods and files to control the machine called Orbit (Andersson, et al., 2017).

## 3.3 Buffer Solutions

During the experiments, several buffers were used. The content of each buffer can be seen in table A.2 in Appendix A. The important with all the buffers is that they have different pH values and the ions in the system only makes sure that the change in pH goes slowly. When the salt was solved in distilled water the pH was measured and then adjusted with hydrochloric acid to the correct pH-value. All the buffers are used for different things, the usage for each buffer can be seen in table A.1 in Appendix A. This table also shows the time each step is run and the flowrate. Each step is also described with its purpose (Healthcare, 2017). All the chemicals for the buffers were purchased from Sigma-Aldrich (Stockholm, Sweden).

## 3.4 Determination of the Concentration in the Raw Extract

The first set of experiments that was made was to see that IgG got adsorbed on the column and then eluted when the pH was changed. With this experiments the concentration IgG in the raw extract was determined by equations 2.1, 3.1 and 3.2. The absorbance, Abs, was calculated by equation 3.1 and the area of the top, $Area_{top}$, and the elution volume given by unicorn for the elution top. The concentration was then calculated using Beer-Lamberts law, equation 2.1 and the $\varepsilon$ is molar absorptivity and was taken from (Scientific, 2013), (Carta & Jungbauer, 2010).

$$Abs = \frac{Area_{top}}{Volume} \tag{3.1}$$

These calculations gave the concentration in the purified IgG solution. To get the start concentration in the raw extract equation 3.2 was used. There $c_1$ is the concentration in the raw extract, $v_1$ the volume that was injected, $c_2$ is the concentration purified IgG solution and $v_2$ the volume of the purified IgG solution.

$$C_1 V_1 = C_2 V_2 \tag{3.2}$$

Since the process at KTH is operated in batch mode and several bottles were used, the concentration shifted between the bottles. All the different concentrations have not been displayed in this report since in the end they were mixed during the breakthrough experiments. The concentration during the breakthrough experiments has been displayed together with each graph.

## 3.5 Determination of the Void and Column Capacity

The first two constants that were determined were $\varepsilon$ and $\varepsilon_c$ for HiTrap MabSelect SuRe 1ml column. It was approximated that the void was the same for both HiTrap MabSelect SuRe and HiTrap MabSelect Prisma A. These two constants were determined by adding 0,3ml solution containing 1g/l blue dextran and 1g/l sodium chloride to the column and measuring the time from inject to when they were detected by the detectors. The blue dextran gave indication on the UV and the Sodium chloride gave indication on the conductivity. During these experiments a solution containing 0,2M sodium chloride was used as a buffer. Since there is a dead time in the pipes from the injection point to the column and from the column to the detectors the same experiment was executed again but this time the column was removed. This was done to compensate for the dead time and thereby see how long time it took to pass through the column. When these times were measured equations 3.3 and 3.4 were used to calculate the constants. To get a better estimation of the constants the same experiment was made three times with different flowrates each time. The flowrates that was used was 0,5ml/min, 1ml/min and

1,5ml/min (Ng, et al., 2012). The $\Delta t$ is the time it took to pass through the column, the F is the flowrate and V the volume of the column. $\varepsilon$ is the total void in the column, $\varepsilon_c$ is the column void and $\varepsilon_p$ is the particle void.

$$\varepsilon_c = \frac{\Delta t_{Dextran} F}{V} \tag{3.3}$$

$$\varepsilon = \frac{\Delta t_{NaCl} F}{V} \tag{3.4}$$

Then the values calculated from equation 3.3 and 3.4 was used to calculate $\varepsilon_p$ by equation 3.5. (Schmidt-Taub, 2015)

$$\varepsilon = \varepsilon_c + (1 - \varepsilon_c) \varepsilon_p \tag{3.5}$$

The maximum amount of protein a column can bind in can be calculated accordingly to equation 3.6. There $q_{max,ml}$ is a value from Ge healthcare of how much 1ml stationary phase can bind in, V the column volume and $\varepsilon_c$ the column void (Healthcare, 2005).

$$q_{max} = q_{max,ml} V \varepsilon_c \tag{3.6}$$

## 3.6 Simulation Work Path

The study started with modeling for a single column. To be able to model a single column several constants and equations were needed. The equations can be seen in chapter 3.9, the boundary conditions in chapter 3.10 and the constants with a description can be seen in Appendix B. The only problem with these constants was that most of them was not given so they had to be estimated out of experiments.

When all the parameters and physical properties was estimated, the only thing remaining was the interaction between the column and the raw extract. To know more about this some more experiments were performed with different gradients between buffer A and C. Gradients were performed from 65% Buffer C at start to 85% C at end. With these experiments the experimental data was saved to use for calibration of the model parameters. At the beginning the constants were taken from article (Ng, et al., 2012) and adjusted to get a moderately fit. Then these made up constants was used as starting value when a calibration was made in MATLAB. The calibration in MATLAB used the function "lsqcurvefit" which calculates the distance between the sum of fsquares of the residuals calibration curve and the simulated curve and then changes the parameters to make this distance as small as possible.

## 3.7 Experimental Work Path

When the raw extract was injected to the column the impurities gave a UV result at approximately 2170 milli absorbance units (mAU) and when the raw extract was injected direct to the UV sensor it gave a result at 2190 mAU. This means that when breakthrough occurs the UV signal increases with <1% which is hard to detect and would probably not get a good breakthrough profile. Because of this it was decided that the raw extract was purified a little at the time and the eluted IgG was saved.

To speed up the purification of IgG from the raw extract it was decided to run the purification by CaptureSMB. To be able to run CaptureSMB four Versatile valves, one injection valve, three inlet valves, three pumps, two UV sensors, one conductivity sensor, one pH sensor and two

columns were used. The flow paths can be seen in figure 3.2. The flow directions and valves positions for each step can be seen in chapter 3.7.1. In figure 3.2 the injection valve, conductivity sensor, pH sensor and inlet valves have been excluded since they are not the main detectors or valves.
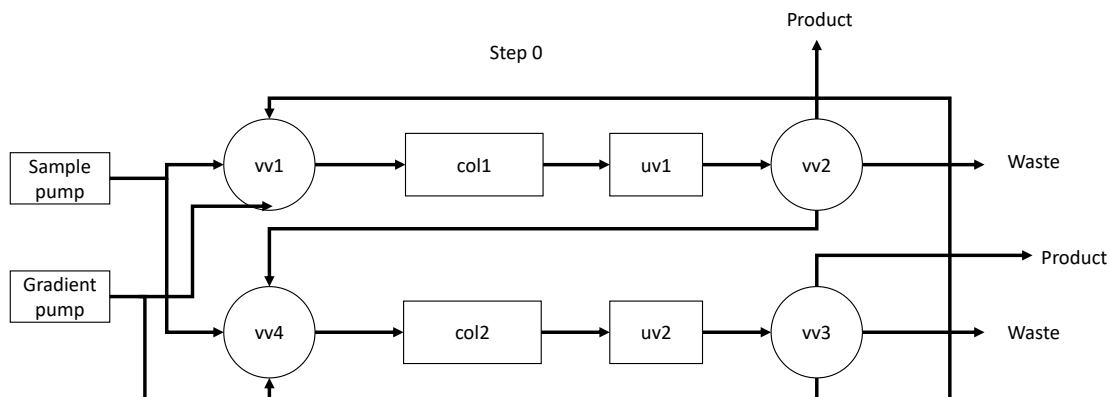


*Figure 3.2. Description of the pipe paths for CaptureSMB.*

The saved purified IgG was injected to the column and when the UV started to increase IgG passed through the column without binding in to the column. When the UV gets to a certain level the injection will stop and then continue accordingly to the steps described in table A.1 in Appendix A.

Breakthrough experiments were made with different columns. As a beginning one 1 ml HiTrap MabSelect SuRe was used and two experiments were made. One with a flowrate at 1ml /min and one with a flowrate at 0.5ml/min. After this the same two experiments was made again but with a 1ml HiTrap MabSelect Prisma A column instead.

Since the breakthrough profiles were slow it was decided to test with another column directly after the first one so it became a 2 ml column. The two ml column had the same diameter as the 1 ml column but was twice as long. Unfortunately this was never done since the IgG denaturized and had to be discarded.

### 3.7.1 CaptureSMBs Different Steps

One cycle of CaptureSMB can be divided into six different steps. The steps have been named to 1, 1,5 2, 3, 3,5 and 4 and will be shown in figure 3.3 to 3.8. In the figures the black flow path represents the flow of raw extract and the grey flow path represent the flow from the gradient pump. The dashed lines are pipes that are not used in that setup and the dotted lines in figure 3.5 and figure 3.8 means that they are only used when product is extracted.
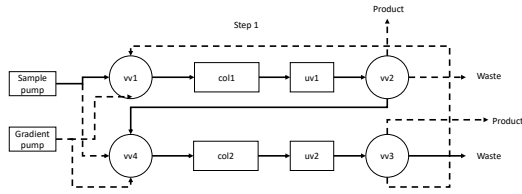
*Figure 3.3. Step 1 in the cycle. Column 1 is loaded and column 2 captures the breakthrough.*
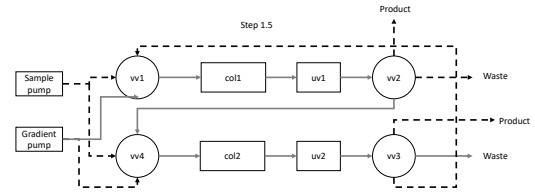


*Figure 3.4. Step 1,5 in the cycle. Washing column one with Buffer A and loads the raw extract from column one to column 2.*
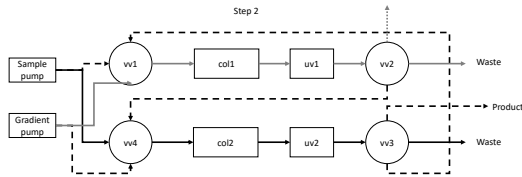


*Figure 3.5. Step 2 in the cycle. Column 1 is eluted, striped, ciped and regenerated and column 2 is loaded.*
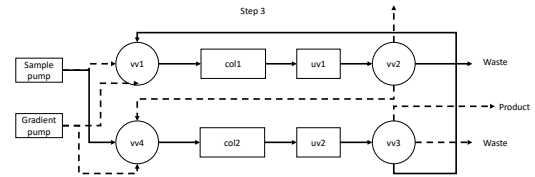


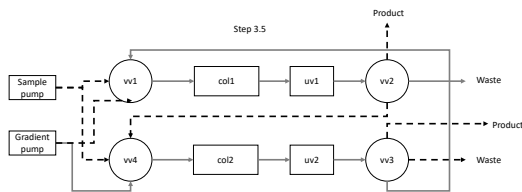*Figure 3.6. Step 3 in the cycle. Column 2 is loaded and column 1 captures the breakthrough.*



*Figure 3.7. Step 3,5 in the cycle. Column 2 is washed and the raw extract from column 2 is loaded to column 1.*
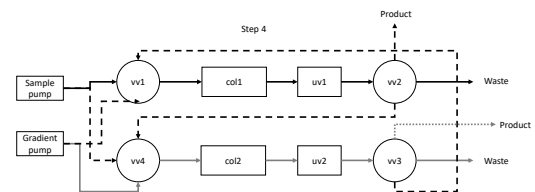


*Figure 3.8. Step 4 in the cycle. Column 2 is eluted, striped, ciped and regenerated and column 1 is loaded.*

## 3.8 Analyzation of Experimental Data

The experimental data was given in two ways from unicorn. The first one was by graphs and the second one as raw data in excel. The excel file given was in the format csv and as text. At this point it was also chosen to resave the raw data with the file format xlsx. The raw data was then read in to MATLAB by the command "xlsread" to be analyzed. In MATLAB the raw data was used for drawing graphs and used for the calibration. The graphs drawn by unicorn was harder to use since they were drawn in color. Most of them has been redrawn in MATLAB to make them in grayscale but some has been used by making them to grayscale in word.

## 3.9 Simulation Model

Equation 3.7 describes the mass transfer inside the column for the mobile phase. Both isothermal adsorption and radial homogeneity has been assumed. The last term in the expression describes the mass transfer through a film between the outside and inside of the particles (Ng,

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et al., 2012). The first term to the right of the equal sign represents the convective term and the second term to the right of the equal sign represents the axial dispersion (Guiochon, et al., 2006). $D_{ax}$ is a coefficient for the axial dispersion, $v_{int}$ interstitial velocity through the column, c the concentration in the bulk, $c_p$ the concentration inside the particles, $k_{film}$ is a mass transport coefficient, $\varepsilon_c$ the column void, t the time and z is a length coordinate along the column (Sellberg, 2018).

$$\frac{\partial c}{\partial t} = - v_{int} \frac{\partial c}{\partial z} + D_{ax} \frac{\partial^2 c}{dz^2} - \frac{1- \varepsilon_c}{\varepsilon_c} \frac{3}{r_p} k_{film} (c - c_p) \tag{3.7}$$

Since an affinity chromatography column has been modeled the salt in the raw extract and in the buffers won't bind in to the column. For salt, the last term from equation 3.7 can be neglected and instead of axial dispersion and interstitial velocity apparent dispersion and apparent velocity is used forming equation 3.8. This equation does not affect the adsorption and is only used to match the times in the simulation to the experimental results (Sellberg, 2018).

$$\frac{\partial c_s}{\partial t} = - v_{app} \frac{\partial c}{\partial z} + D_{app} \frac{\partial^2 c}{dz^2} \tag{3.8}$$

The diffusion into the stationary phase pores is described by equation 3.9. This equation is only dependent on the outer film adsorption kinetics. Where $r_p$ is the particle radius (Sellberg, 2018).

$$\frac{\partial c_p}{\partial t} = \frac{3}{r_p} k_{film} (c - c_p) - \frac{\partial q}{\partial t} \tag{3.9}$$

The equation that determines the separation is the adsorption isotherm (Sellberg, 2018). The adsorption isotherm used in this study can be seen in equation 3.10 (Ng, et al., 2012). Where q is the concentration in the stationary phase, q* is the concentration in the stationary phase at equilibrium and $k_m$ is a lumped mass transfer coefficient.

$$\frac{\partial q}{\partial t} = k_m (q^* - q) \tag{3.10}$$

Equation 3.11 describes the lumped mass transfer coefficient used in equation 3.10. $S_1$ is the saturation dependent kinetic constant, $S_2$ is the saturation dependent order and $k_{max}$ is the maximum lumped mass transfer coefficient (Ng, et al., 2012).

$$k_m = k_{max} \left( S_1 + (1 - S_1) \left( 1 - \frac{q}{q_{max}} \right)^{S_2} \right) \tag{3.11}$$

To describe the adsorption isotherm in the column a Langmuir isotherm has been chosen. This Langmuir adsorption isotherm is modified by the pH and can be seen in equation 3.12. The model was only used for the IgG and not for the impurities. This because affinity chromatography is based on that only the target protein bind in to the column and the rest only passes through. The C represents the concentration of IgG in the mobile phase, the pH is the pH in the solution, $pH_{ref}$ the pH in the solution injected, the $K_A$ is association equilibrium constant, the n the pH dependent equilibrium order and $q_{max}$ is the maximum amount bound to the stationary phase (Ng, et al., 2012).

$$q^* = \frac{q_{max} K_A \left(\frac{pH}{pH_{ref}}\right)^n C}{1 + K_A \left(\frac{pH}{pH_{ref}}\right)^n C}$$ (3.12)

Equation 3.13 shows how the apparent axial dispersion coefficient has been defined by an empirical correlation. There $A_{app}$ and $B_{app}$ comes from a modified van Deemter expression and $A_{app}$ is the coefficients for eddy diffusion and $B_{app}$ is for mass transfer resistance, F is the flow, A is the cross-section area of the column and $d_p$ is the particle diameter (Ng, et al., 2012).

$$D_{app} = \frac{F\,d_p}{2\,A}\left(A_{app} + B_{app}\,\frac{F}{A}\right)$$ (3.13)

The axial dispersion in the column are described by equation 3.14. There $v_{int}$ is the interstitial velocity, $d_p$ the particle diameter and the Pe Peclet number (Schmidt-Taub, 2015). The Peclet number has been set to a standard value of 1 since it is a packed bed with macromolecules. (Borg, et al., 2014)

$$D_{ax} = \frac{V_{int}\,d_p}{Pe}$$ (3.14)

$V_{app}$ describes the apparent velocity in the column and was calculated with equation 3.15. (Sellberg, et al., 2017)

$$V_{app} = \frac{F}{\varepsilon\,A}$$ (3.15)

$V_{int}$ describes the interstitial velocity in the column and was calculated by equation 3.16 (Schmidt-Taub, 2015) .

$$V_{int} = \frac{F}{\varepsilon_c\,A}$$ (3.16)

## 3.10 Boundary Conditions

Initially it is assumed that the column only contains solvents and therefore is empty from proteins both in the stationary and mobile phase which can be seen in equations 3.17 and 3.18 (Ng, et al., 2012)

$$c(t = 0, z) = 0$$ (3.17)

$$c_p(t = 0, z) = 0$$ (3.18)

Other boundary conditions used are for the inlet and outlet. For the inlet, a Dankwerts condition is used and for the outlet is a Neumann condition. Both the inlet conditions and outlet conditions can be seen in equations 3.19 and 3.20 (Ng, et al., 2012).

$$c|_{z=0} = c_{init}(t)$$ (3.19)

$$\left.\frac{\partial c}{\partial z}\right|_{z=L} = 0$$ (3.20)

## 3.11 Simulation Approach

To be able to solve equations 3.7 to 3.12 with conditions accordingly to equation 3.17 to 3.20 in MATLAB R2016b the ordinary differential equations solver ode15s was used. The partial differential equations were discretized using the method of lines approach using 100 grid points. The spatial derivate in the partial differential equations (PDE) has to be discretizes by using a Finite Volume Method (FVM). (Sellberg, 2018) The first and second order derivate that was used can be seen in equations 3.21 and 3.22. For the first order a two-point backward difference were used for the estimation and for the second derivate a three-point central difference were used for the estimation (Ramirez, 1997).

$$\frac{\partial c}{\partial z} = \frac{c_i - c_{i-1}}{h} \tag{3.21}$$

$$\frac{\partial^2 c}{\partial z^2} = \frac{c_{i+1} - 2c_i + c_{i-1}}{h^2} \tag{3.22}$$

# 4 Result and Discussion

## 4.1 Experimentally Determined Parameters

The experiments and calculations for calculating the concentration in one of the bottles with raw extract and the three different epsilons for HiTrap MabSelect SuRe can be seen in Appendix C and Appendix D respectively. The result of the calculations is also shown in table 4.1.

*Table 4.1. Experimentally determined parameters.*

| Parameter | Value |
|---|---|
| $\varepsilon_c$ | 0.380 |
| $\varepsilon_p$ | 0.940 |
| $\varepsilon$ | 0.963 |
| concentration | 0.214 g/l |

## 4.2 Parameters Determined by Calibration

During the calibrations for simulation to experimental data the constants in table 4.2 were set to a specific value. They were set to a specific value because it was observed by a sensitivity analysis, that is shown in Appendix E, that even if these constants shifted the changes was marginal. The graphs in Appendix E is for the same experimental data as figure 4.2 but with change of simulation parameters. A sensitivity analysis was also done for $k_a$ and n in Appendix E but these deviated a lot from the experimental data and therefor it was decided to calibrate on these two. All the experiments in this section were executed on a HiTrap MabSelect SuRe 1ml column.

*Table 4.2. Parameters that was set to a specific value after the sensitivity analysis. The parameters were calibrated by hand to these values with a starting point from (Ng, et al., 2012).*

| Parameter | Value |
|---|---|
| $S_1$ | 0.76 |
| $S_2$ | 4 |
| $k_{film}$ | $1 \cdot 10^5$ |
| $k_{max}$ | $1.6 \cdot 10^2$ |

### 4.2.1 Parameters Determined with Gradient

The simulated result of fitting a simulation to an experimental data can be seen in figure 4.1. The parameters that the curve was calibrated on can be seen in table 4.3. As can be seen the curve fits quite well to the experimental data which is good since that means that the model can predict when the product elutes. In this case the gradient went between 65% C and 85% C.
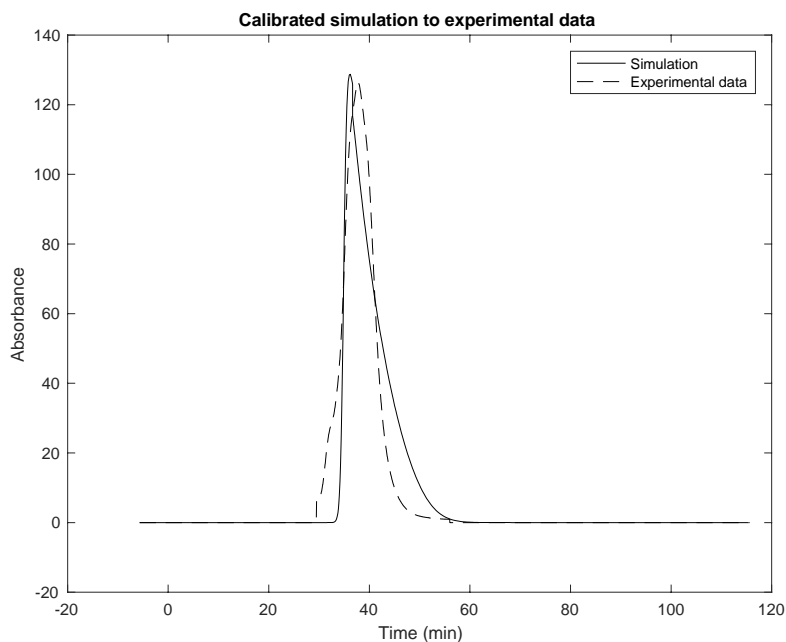


*Figure 4.1. Calibrated simulation to fit the experimental data for a gradient from 65% buffer C to 85% buffer C. During the gradient buffer C was mixed with Buffer A. The concentration in the raw extract was 0,170mg/ml.*

*Table 4.3. Calibrated parameter values for the simulation with a buffer gradient.*

| Parameter | Value |
|---|---|
| $K_a$ | $10.2 \cdot 10^5$ |
| n | 10.3 |

### 4.2.2 Parameters Determined without Gradient

The result from section 4.2.1 was used as starting points when the parameters for elution without gradient was determined. The simulated data and the experimental data can be seen in figure 4.2. The parameters for this simulation can be seen in table 4.4. Also, here we got a good fit between the simulation and the experimental data.
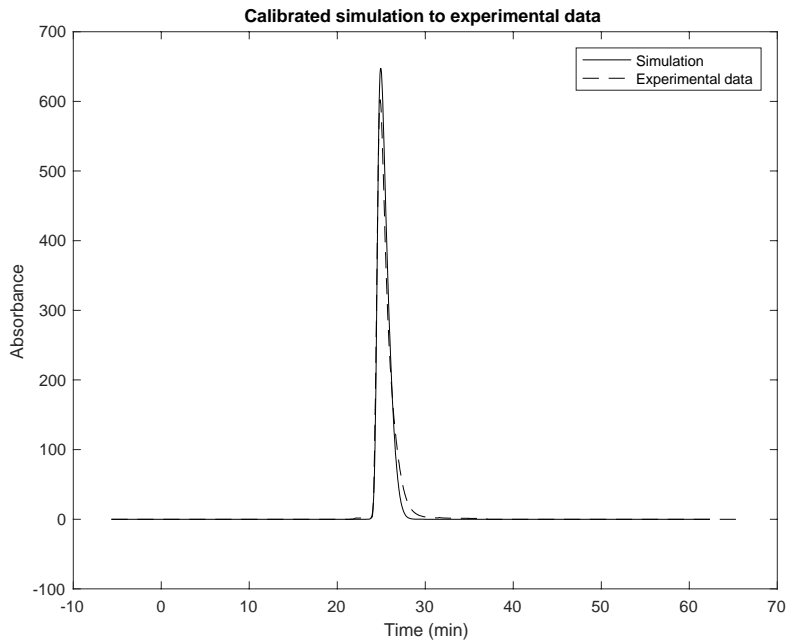
*Figure 4.2. Calibrated simulation to fit the experimental data. The concentration in the raw extract was 0,170mg/ml.*

*Table 4.4. Calibrated parameter values to fit the experimental data.*

| Parameter | Value |
|-----------|-------|
| $K_a$ | $5.17 \cdot 10^5$ |
| n | 11.6 |

The parameters that was calibrated on for the two experiments shown in sections 4.2.1 and 4.2.2 are in the range of the same tenfold. If a new set of experiments were made on the same way and they were calibrated again they would probably be slightly different from the results presented in this study. That depends on several things, such as the raw extract may not have been completely homogenous or the proteins may take another way through the column which will change the elution time slightly. This means that the results presented in this study would be a good starting point for the calibration but it would probably give a slightly different value.

## 4.3  Breakthrough

The reason that a high slope on a breakthrough curve is desired is because of as soon as it starts to break through some of the desired product goes to the waste. This means that product will be lost and the yield will be lowered. The solution that was injected during the breakthrough experiments in this study wasn't completely pure which can be seen that the UV doesn't start at zero. The height of the step in the beginning is directly proportional to the amount impurities. During all the breakthrough experiments there were an offset baseline. The height of the baseline changed a bit during the different experiments but was around 15. The height of the base line can be seen by the step in the beginning of each experiment.

### 4.3.1 HiTrap MabSelect SuRe

Breakthrough experiments were made at different flowrates. The first two breakthrough experiments that were successfully made were at flowrate 0.5 and 1 ml/min. The graphs from these experiments can be seen in figure 4.3 and 4.4 where the slope of the breakthrough curve is very low. This means that the amount IgG that leaks through the column increases during the time. As in this case the slope is very low which is unwanted because it means low productivity or low yield. The productivity will decrease if the load is stopped when it starts to leek through the column since only parts of the column is filled with IgG. The yield will decrease if IgG leeks through the column since it goes to waste. Because of this it needs to be optimized. The two spikes that can be seen in figure 4.3 is a disturbance because of the injection ended and was then started again without the elution, strip, CIP and regeneration in between.

For the breakthrough curve with a flow of 0,5ml/min shown in figure 4.3 it can be seen that for the beginning there is a starting time where zero IgG leaks through. For the breakthrough curve with a flowrate at 1ml/min it can be seen in figure 4.4 that it starts to leak through immediately after the injection started.
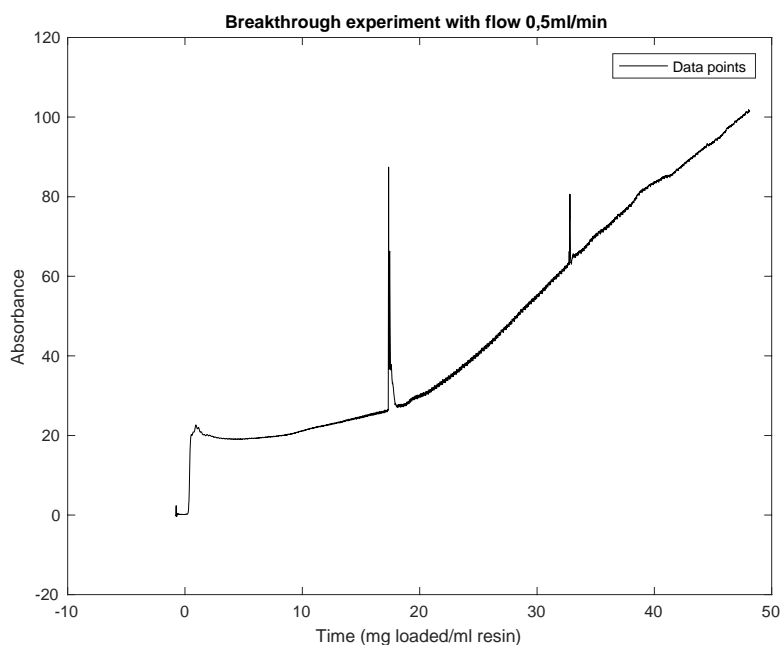


*Figure 4.3. Breakthrough experiment with a flowrate at 0.5ml/min with a HiTrap MabSelect SuRe 1 ml column. The concentration during the experiment was 0,170mg/ml.*
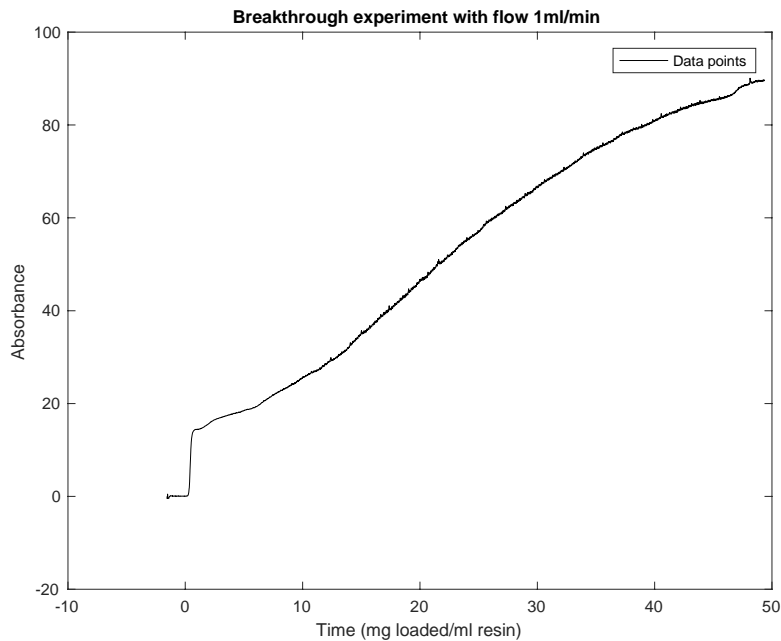
*Figure 4.4. Breakthrough experiment with a flowrate at 1ml/min with a HiTrap MabSelect SuRe 1 ml column. The concentration during the experiment was 0,170mg/ml.*

### 4.3.2   HiTrap MabSelect Prisma A

Breakthrough experiments was made with a HiTrap MabSelect Prisma A column also. To be able to compare the two columns, these experiments was made at the same flowrate as HiTrap MabSelect SuRe column. The two breakthrough curves can be seen in figure 4.5 and figure 4.6. As can be seen when the figures are compared the breakthrough curve in figure 4.5 have a greater slope compared to figure 4.6. This means that the column is filled more evenly and when the column is filled it leaks through. It can also be seen that the time from start to the breakthrough occurs are longer for the experiment with a flowrate at 0,5ml/min compared for the experiment with a flowrate at 1ml/min.
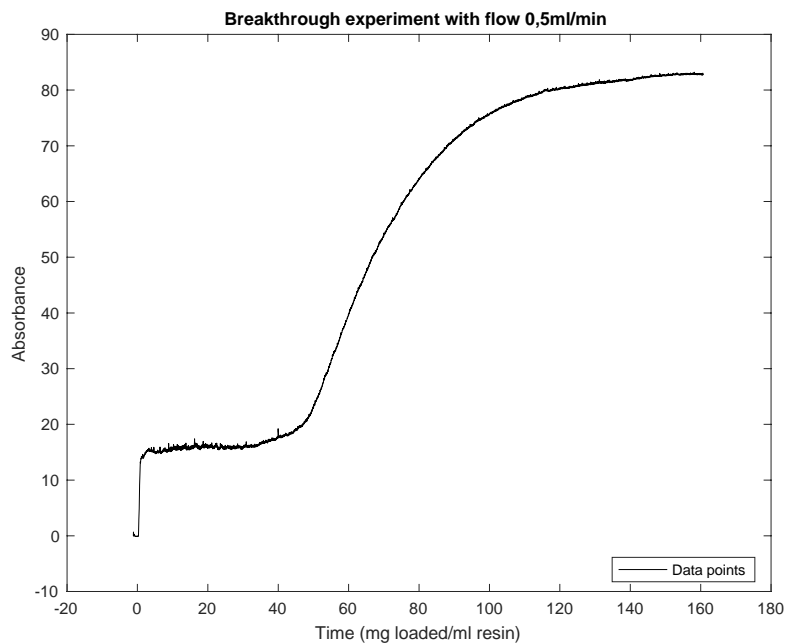
*Figure 4.5. Breakthrough experiment with a flowrate at 0.5ml/min with a HiTrap MabSelect Prisma A 1 ml column. The concentration during the experiment was 0,251mg/ml.*
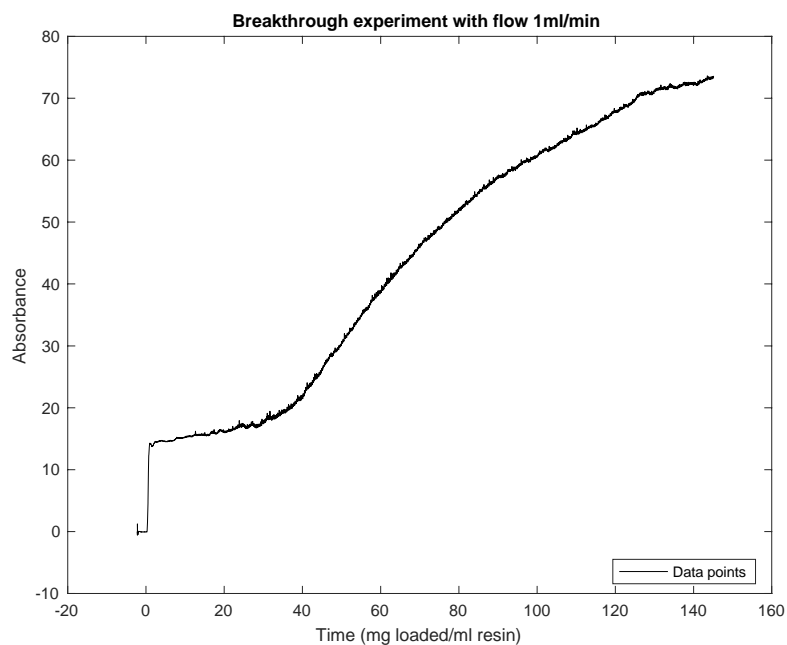


*Figure 4.6. Breakthrough experiment with a flowrate at 1ml/min with a HiTrap MabSelect Prisma A 1 ml column. The concentration during the experiment was 0,244mg/ml.*

### 4.3.3    Comparison between HiTrap MabSelect SuRe and HiTrap MabSelect Prisma A

From the graphs presented in figure 4.3 to figure 4.6 it is possible to calculate the maximum capacity for the columns. This is done by an assumption that the breakthrough curve is a straight line. With that assumption the midpoint of the breakthrough curve is taken on the x-axis as the maximum capacity. This because then it is the same amount that has leaked through without binding in to the column as there is free capacity in the column.

The main differences between the HiTrap MabSelect SuRe and HiTrap MabSelect Prisma A are the amount IgG they can absorb. The maximum capacity were calculated by taking the average value for the two experiments. For HiTrap MabSelect SuRe the maximum capacity is approximately 35mg IgG/ml column and for HiTrap MabSelect Prisma A the maximum capacity is approximately 80mg IgG/ml column. The difference in capacity is probably because of the difference in particle diameter in the two columns. With a smaller particle diameter the surface area and the pore depth will decrease. The other side with smaller particle is that there will be more particle in the same volume compared with larger particles. Also the gaps between the particles will decrease with smaller particles. These things together probably give a bigger total surface area for HiTrap MabSelect Prisma A columns compared to HiTrap MabSelect SuRe columns. With smaller particle depth the pore will get filled faster. Since the change in concentration in the particles depends on the diffusion only the surface area will be filled a lot faster than the pores. Because of this it is good with pores that aren't too deep since it will be the limiting factor in filling the column.

An uncertainty with these experiments are that the solution injected has different concentration.

### 4.3.4    Comparison between 0,5ml/min and 1ml/min

For the comparison between 0,5ml/min and 1ml/min figure 4.3 and 4.5 are looked at together and figure 4.4 and 4.6 together. The biggest difference between the two flowrates are that for 0,5ml/min the time from injection to breakthrough occurs are longer. This is probably because with the lower flowrate the residence time will increase. This means it will take longer time to pass through the column which gives the IgG more time to react with the pore surface or diffuse in to the pores.

This indicates that a lower flowrate will give a greater slope on the breakthrough curve. The downside with a lowered flowrate is that the productivity will decrease since it takes longer time to load the IgG to the column. To prove this about the breakthrough curve more experiments are needed. For example would it be a good idea to investigate this thoroughly by doing the same experiments but with other flowrates.

## 4.4 CaptureSMB

During this study CaptureSMB was studied and tested experimentally. During this experiment three complete cycles were performed. Each step in the cycle were set to a specific time which can be seen in table A.3 in Appendix A. In figure 4.7 the experimental data are presented for the two UV signals. Figure 4.8 is a cut from figure 4.7 and is a bit more than one cycle. In figure 4.8 the most important steps are marked. The horizontal line with two arrows is the length of one cycle. The two boxes are for loading, the first one for loading column one and the second one for column two. During the loading step the other column are placed behind the column being loaded to capture the breakthrough. The arrows indicates when the columns are eluted. The first and third arrow are when column two are eluted and the second one when column one are eluted. Under each step there is a letter and a number. The number indicates if it is column one or two and the letter indicates if it is loading or elution. The baseline during this experiment was about 2500 for UV 1 and 2700 for UV 2.
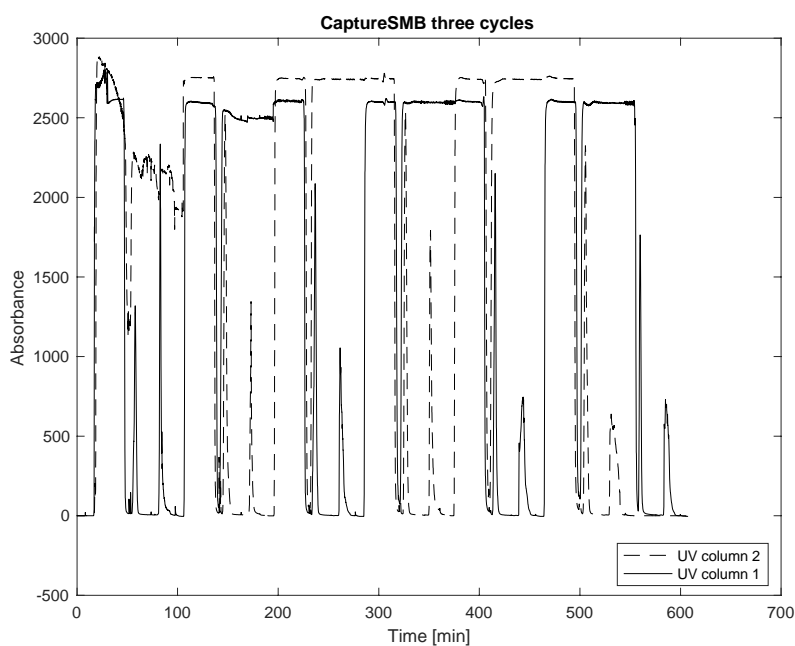


*Figure 4.7. The UV signals for three cycles of CaptureSMB. The baseline during the load section was at approximately 2500 mAU for column 1 and 2700 mAU for column 2.*
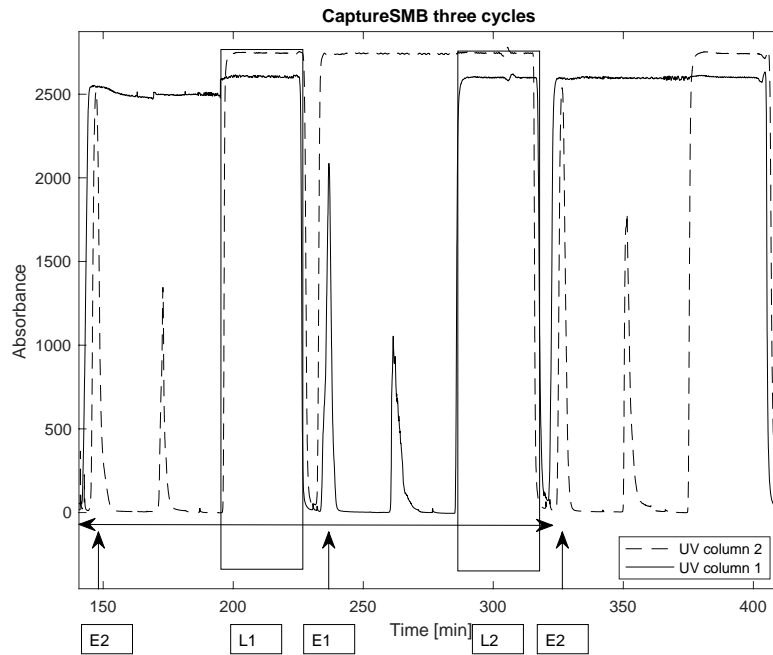
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*Figure 4.8. Magnification of a little more than one cycle of Capture SMB. The horizontal line with two ar-rows is the length of one cycle. The two boxes are for loading, the first one for loading column one and the second one for column two. During the loading step the other column are placed behind the column being loaded to capture the breakthrough. The arrows indicates when the columns are eluted. The first and third arrow are when column two are eluted and the second one when column one are eluted. Under each step there is a letter and a number. The number indicates if it is column one or two and the letter indicates if it is loading or elution.*

All the figures in section 4.4 shows that the UV detector after the second column gives a greater detection than the UV detector after the first column. This depends probably on that the UV detectors have been differently calibrated. For this experiments it does not matter but if pure IgG were used and breakthrough from the columns were used as online controlling it would be a problem. This process that has been carried out could probably be optimized quite much. To start the optimization, the breakthrough curve needs to be optimized. The second step is to fill the second column as much as possible without getting breakthrough during the elution and regeneration of the first column. Another way of optimizing the CaptureSMB is to operate as many cycles as possible after each other since there is both a starting phase and an end phase of each run. The starting phase consists of equilibrating the columns and the end phase elutes and regenerates the first column since it has been used as a capture during the filling of the second column.

Another thing to do in the future is to operate CaptureSMB with pure IgG. This because all the impurities makes it impossible to detect breakthrough and therefore cannot the yield be calculated. Another thing when the CaptureSMB is optimized is to calculate the productivity. Since CaptureSMB only was tested the productivity was never calculated in this study.

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## 4.5 Summary

During this study several experiments and simulations has been done. Everything started with some basic experiments and a literature study. From the literature study a model was assembled and fitted to the experimental data. The next step was to do some more experiments with and without a elution gradient. The model was then calibrated on these new experimental data. The next step in this work was to investigate the breakthrough curve. During the study four different breakthrough curves were investigated. Due to lack of time at the end of this study and denaturated of protein the breakthrough curve wasn't satisfyingly investigated. Since the proteins denaturation and precipitated all the test solution was discarded. Since it had to be discarded and it was so little time left of the study it was decided to not purify any more during this study. Mostly it was the breakthrough profiles that got effected of this and the comparison between the columns since the plan was to use two 1ml columns after each other and see how that effected the breakthrough profile. The reason for the denaturation was probably that the protein had been in the fridge too long and also because of the constant heating to room temperature during each run. The last part in this study was the investigation of CaptureSMB. CaptureSMB was successfully tested experimentally in the laboratory there three cycles were performed.

# 5 Conclusion

The investigation of affinity chromatography was successfully made. The process was successfully simulated with a lumped rate model dependent on pH. In the end it was found by a sensitivity analysis that the model was only dependent on two different parameters, the pH dependent equilibrium order and an association equilibrium constant. The model used to predict the elution didn't work as a model for the breakthrough curve.

Also the breakthrough curve was investigated. It was found that with a lower flowrate the breakthrough curve gets a greater slope. It was also found out that HiTrap MabSelect Prisma A has a greater capacity than HiTrap MabSelect SuRe.

During this study CaptureSMB was investigated and tested. The investigation gave an insight on how to operate and run CaptureSMB. The experimental test showed that it was possible to operate as suggested in this report even though it wasn't optimized.

# 6 Future Work

There is still a lot to do in the future in this area. Some of the things that can be more investigated can be divided into three categories, simulation parameters, breakthrough and CaptureSMB

## 6.1 Simulation Parameters

The simulation parameters presented in this report is only valid for the purification of IgG with the same buffers that was used in this study. Some things that could be done in this area in the future is to investigate how much the buffers affect the parameters. As a result of this it would be possible to give parameters with a confidence interval independent of the buffers.

Another thing that can be investigated more is the model used in this study. The model presented in this study fits quite well for the elution but don´t fit the breakthrough curve. Would it be possible to get a model that fits both?

## 6.2 Breakthrough

In this study only the breakthrough curve has been analyzed and tested with some different parameters. To be able to optimize the breakthrough curve more experiments are needed and also simulation of the breakthrough curve. Suggestion on following experiments are to change the column length, change the flowrate and it could also be of interest to see if changes in concentration will affect the breakthrough curve.

## 6.3 CaptureSMB

Further work on CaptureSMB is dependent on the breakthrough profile. If the breakthrough profile gets optimized that can be used as a part to optimize the CaptureSMB. Another part in the further development of CaptureSMB is the usage of valves. Since there is a limit of valves on a machine the setup might need to be changed to fit the rest of the process.

# 7 References

Aldrich, S., 2018. *IgG from Human Serum.* [Online] Available at: https://www.sigmaaldrich.com/catalog/product/sigma/i2511?lang=en&region=SE [Accessed 25 5 2018].

Andersson, N. et al., 2017. *Desing and control of integrated chromatography column sequences,* Lund: Wiley Online Library.

Angarita, M. et al., 2015. Twin-column CaptureSMB: A novel cyclic process for protein A affinity chromatography. *Journal of chromatography A*, 10 April, pp. 85-95.

BioProcess, 2017. *MabSelectTM Prisma A,* Uppsala: GE Healthcare .

Bio-Sciences, 2007. *HiTrap MabSelect SuRe HiTrap MabSelect HiTrap MabSelect Xtra,* Uppsala: GE Healthcare.

Bio-Sciences, G. H., 2017. *Äkta pure user manual,* Uppsala: GE Healthcare.

Borg, N. et al., 2014. Modeling and robust pooling design of a preparative cation-exchange chromatography step for purification of monoclonal antibody monomer from aggregates. *Journal of Chromatography,* Volume 1359, pp. 170-181.

Cabrera, K., Brünner, W. & Müller, E., 2012. Liquid Chromatography, 4. Affinity Chromatography. In: *Ullmans Encyclopedia of industrial chemistry.* Weinheim: Wiley-VCH.

Carta, G. & Jungbauer, A., 2010. *Protein Chromatography Process Development and Scale-Up.* Weinheim: Wiley-VCH.

Corporation, P., n.d. Buffers for Biochemical Reactions. In: *Protocols & applications guide.* s.l.:Promega Corporation.

Guiochon, G., 2012. Basic Principles of chromatography. In: *Ullmanns Encyclopedia of industrila chemistry.* Weinheim: Wiley-VCH.

Guiochon, G., Felinger, A., Shirazi, D. G. & Katti, A. M., 2006. *Fundamentals of preparative and nonlinear chromatography.* 2 ed. San Diego: Elsevier Inc.

Hage, D. S., 2014. Chromatography, Affinity. In: *Kirk-Othmer Encyclopedia of Chemical technology, 1.* s.l.:John Wiley & Sons, Inc.

Healthcare, G., 2005. *HiTrap MabSelect SuRe 1 ml and 5 ml.* Uppsala: GE Healthcare Bio-science AB.

Healthcare, G., 2017. *Evaluation of Protein A resin lifetime during extensive use (overloading) in continuous chromatography,* s.l.: GE Healthcare Bio-Sciences AB.

Jandera, P. & Henze, G., 2012. Liquid Chromatography, 1. Fundamentals, History, Instrumentation, MAterials. In: *Ullmans encyclopedia of industrial chemistry.* Weinheim: Wiley-VCH.

Jandera, P. & Lembke, P., 2012. Liquid Chromatography, 2. Methods, Programmed and Coupling Techniques. In: *Ullmans Encyclopedia of industrial chemistry.* Weinheim: Wiley-VCH.

Kaiser, M. A. & Dybowski, C., 2000. Chromatography. In: *Kirk-othmer Encyclopedia of chemical technology.* s.l.:John Wiley & Sons, Inc.

Lembke, P., 2012. Liquid Chromatography, 3. Supercritical Fluid Chromatography. In: *Ullmans encyclopedia of industrial chemistry.* Weinheim: Wiley-VCH.

Mallery, D. L. et al., 2010. *Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21),* s.l.: National Academy of Sciences.

Ng, C. K. et al., 2012. Design of high productivity antibody capture by protein A chromatography using an integrated experimental and modeling approach. *Journal of chromatography*, 14 June, pp. 116-126.

Nobelprize.org, n.d. *The Nobel Prize in Chemistry 1952.* [Online] Available at: https://www.nobelprize.org/nobel_prizes/chemistry/laureates/1952/ [Accessed 12 3 2018].

Ramirez, F. W., 1997. *Computational Methods for Process Simulation.* Second Edition ed. Oxford: Butterworth-Heinemann.

Schmidt-Taub, H., 2015. *Preparative Chromatography of fine Chemicals and Pharmaceutical Agents.* Weinheim: Wiley-VCH.

Scientific, T., 2013. *Extinction Coefficients A guide to understanding extinction coefficients, with emphasis on spectrophotometric determination of protein concentration,* Rockford: Thermo Scientific.

Sellberg, A., 2018. *Open-Loop Optimal Control of Chromatographic Seperation Processes.* Lund: Department of chemical Engineering Lund University.

Sellberg, A., Andersson, N., Holmqvist, A. & Nilsson, B., 2017. Development and Optimization of a Single Column Analog Model for a Multi-Column Counter-Current Solvent Gradient Purification Process. *Computer Aided Chemical Engineering,* Volume 40, pp. 187-192.

# 8 Appendices

## 8.1 Appendix A: Buffers

*Table A.1. Description of when the different buffers is used and their purpose.*
*\*Only used for the experiments with a gradient.*

| Step | Flowrate (ml/min) | CV | Buffer | description |
|---|---|---|---|---|
| Equilibration | 1 | 5 | A | Preparing the column and filling it up with buffer A. |
| Load | 1 | - | - | Raw extract loads to the column |
| Post load | 0.4 | 2 | A | Washes of the remaining raw extract. |
| Wash 1 | 1 | 3 | A | First wash so there is only buffer A and IgG left |
| Wash 2 | 1 | 1 | B | Second Wash. Elutes if anything except IgG has stuck on the column |
| Elution gradient* | 0.5 | 7.5 | 65%C -> 85%C, <br><br> 35%A -> 15%A | Elutes the IgG |
| Elution 1* | 0.5 | 4 | 85%C, 15%A | Makes sure that all IgG elutes. |
| Elution 2 | 0.5 | 5 | C | Elutes the IgG. *Makes sure that all IgG elutes. |
| Strip | 1 | 1 | D | Washes out everything even if it has stuck harder than IgG |
| Cleaning in place (CIP) | 0.33 | 5 | E | Regenerates the column |
| Re-equilibration | 1 | 5 | A | Prepare the column for the next load. |

*Table A.2. The contents of the buffers used for CaptureSMB.*

| Chemical | Chemical formula | Concentration |
|---|---|---|
| Buffer A pH 7.4 | | |
| Sodium chloride | NaCl | 137mM |
| Potassium chloride | KCl | 2.7mM |
| Disodium phosphate | $Na_2HPO_4$ | 10mM |
| Monopotassium phosphate | $KH_2PO_4$ | 1.8mM |
| | | |
| Buffer B pH 6 | | |
| Sodium acetate | $C_2H_3NaO_2$ | 50mM |
| | | |
| Buffer C pH 3.5 | | |
| Sodium acetate | $C_2H_3NaO_2$ | 50mM |
| | | |
| Buffer D pH 2.9 | | |
| Acetic acid | $CH_3COOH$ | 100mM |
| | | |
| Buffer E pH 13 | | |
| Sodium hydroxide | NaOH | 100mM |

*Table A.3. Description of how CaptureSMB were operated and when each buffer were used.*

| Step | Flowrate (ml/min) | CV | Buffer | description |
|---|---|---|---|---|
| Equilibration | 1 | 5 | A | Preparing the column and filling it up with buffer A. |
| Load | 1 | 30 | - | Raw extract loads to the column |
| Post load | 0,4 | 2 | A | Washes of the remaining raw extract. |
| Wash 1 | 1 | 3 | A | First wash so there is only buffer A and IgG left |
| Wash 2 | 1 | 1 | B | Second Wash. Elutes if anything except IgG has stuck on the column |
| Elution | 0.5 | 5 | C | Elutes the IgG. |
| Strip | 1 | 1 | D | Washes out everything even if it has stuck harder than IgG |
| Cleaning in place (CIP) | 0.33 | 5 | E | Regenerates the column |
| Re-equilibration | 1 | 5 | A | Prepare the column for the next load. |

## 8.2 Appendix B: Variables and Description

*Table B.1. All the parameters with description and values.*

* (Bio-Sciences, 2007), ** (BioProcess, 2017)

| Symbol | Description | Value | Unit |
|---|---|---|---|
| A | Column cross-section area of the column | 0.38 | $cm^2$ |
| $A_{app}$ | Van Deemter eddy diffusion coefficient | 4.8 | - |
| Area | Area under the absorbent curve | - | - |
| $B_{app}$ | Van Deemter mass transfer resistance coefficient | 17 | - |
| c | Concentration | - | $g/m^3$ |
| $c_p$ | Concentration in the particle | - | $g/m^3$ |
| $D_{app}$ | Apparent axial dispersion | - | $m^2/min$ |
| $D_{ax}$ | Axial dispersion in the column | - | $m^2/min$ |
| $d_c$ | Column diameter | 7 | mm |
| $d_{c,\text{HiTrap MabSelect SuRe}}$ | Particle diameter | 85* | µm |
| $d_{c,\text{HiTrap MabSelect Prisma A}}$ | Particle diameter | 60** | µm |
| $\varepsilon$ | Total column void | 0.96 | - |
| $\varepsilon_{Abs}$ | Molar absorptivity | 1.4 | - |
| $\varepsilon_c$ | Column void | 0.38 | - |
| $\varepsilon_p$ | Particle void | 0.94 | - |
| F | Flow | - | $m^3/min$ |
| h | Distance between grid points | - | - |

| $K_A$ | Association equilibrium constant | $5.17 \cdot 10^5$ | $m^3/g$ |
|---|---|---|---|
| $k_{film}$ | Film mass transfer coefficient | $1 \cdot 10^5$ | m/min |
| $k_m$ | Lumped mass transfer coefficient | $1.6 \cdot 10^2$ | $min^{-1}$ |
| $k_{max}$ | Maximum lumped mass transfer coefficient | See table 4.2 | $min^{-1}$ |
| l | Cuvette length | 0.2 | cm |
| L | Column length | 2.5* | cm |
| n | pH dependent equilibrium order | 11.6 | - |
| N | Number of grid points | 100 | - |
| Pe | Peclet number | 1 | - |
| pH | Buffer pH-value | - | - |
| $pH_{ref}$ | Reference value of pH | 7.2 | - |
| q | Concentration adsorbed by the stationary phase | - | $g/m^3$ |
| q* | Concentration adsorbed by the stationary phase at equilibrium | - | $g/m^3$ |
| $q_{max}$ | Maximum amount adsorbed by the stationary phase | - | g |
| $q_{max,ml}$ | Maximum amount adsorbed by 1 ml of the stationary phase | 30* | mg |
| r | Column radius | 3.5* | mm |
| $r_p$ | Particle radius | | μm |

| $S_1$ | Saturation dependent kinetic constant | 0.76 | - |
|---|---|---|---|
| $S_2$ | Saturation dependent kinetic order | 4 | - |
| t | Time | - | min |
| $\Delta t_{dextran}$ | Time for dextran to go through the column | 0.38 | min |
| $\Delta t_{NaCl}$ | Time for NaCl to go through the column | 0.96 | min |
| $V_{app}$ | Apparent velocity in packed column | - | m/min |
| $V_{int}$ | Interstitial velocity in packed bed | - | m/min |
| Volume | Volume of the absorbent area | - | $m^3$ |
| z | Axial coordinate | - | m |

## 8.3 Appendix C: Calculation of Concentration in Raw Extract

The experimental run log from unicorn, made to estimate the concentration in the raw extract can be seen in figure C.1. For this experiment an 1ml HiTrap MabSelect SuRe column has been used. In the graph the detection of UV, conductivity and pH can be seen where the UV is the most interesting one. As can be seen the UV detector gives a detection approximately between 6 and 16 ml and this is from the injection since all the impurities eluates directly and the IgG remain in the column. Then when the pH gets lower the IgG eluates after 29,6 ml. Also table C.1 is of interest since it shows the concentration, time, area and volume of the top which eluted after 29,6 ml. The extinction coefficient in table C.1 was added manually to the table in unicorn and then unicorn calculates the concentration of the top by using equations 2.1 and 3.1. Since it is not the concentration in the pure IgG that is of interest equation 3.2 was used to calculate the concentration in the raw extract which was calculated to 0,214 mg/l for this batch.
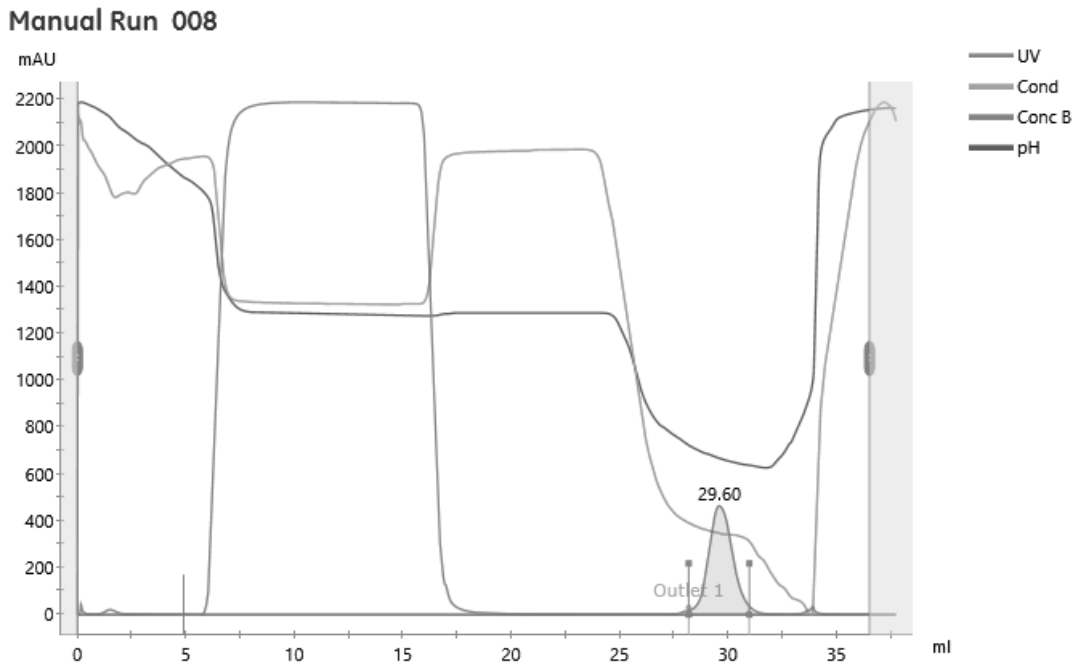


*Figure C.1. Shows the experimental run log for the experiment made to estimate the concentration in the raw extract.*

*Table C.1. The data from the experiment made to estimate the concentration in the raw extract.*

| Peak | Retention (ml) | Area (ml·mAU) | Extintion coefficient (mg/(ml·cm)) | Volume (ml) | Amount (mg) | Concentration (mg/ml) |
|------|---------------|---------------|-----------------------------------|-------------|-------------|----------------------|
| A | 29.601 | 598.8 | 1.40 | 2.799 | 2.139 | 0.764 |

## 8.4 Appendix D: Calculation of the Column Void

To calculate the three different voids for a 1ml HiTrap MabSelect SuRe column some experiments were made. The results from the experiments can be seen in figure D.1, D.2, D.3, D.4, D.5 and D.6 and tables D.1 and D.2. For the first two set of tops the flow was 0,5ml/min, the second 1ml/min and for the third 1,5ml/min. The first of the tops in each set is for UV and the second one is for conductivity. Figure D.1 and D.2 are with the column and figure D.3, D.4, D.5 and D.6 are without column. The injections for all the experiments was made after 9,8ml, 15,11ml and 20,41ml respectively. The difference between figure D.1 and D.2, D.3 and D.4 and between D.5 and D.6 is which detection form that is used. For figures D.1, D.3 and D.5 the detection is made by UV and the retention time can be seen both in the figures and in table D.1 and D.2. The same is for the conductivity but then it is figure D.2, D.4 and D.6. As can be seen there is six figures in this appendix but four should be enough. This is because in figure D.3 and D.4 the first top shouldn't be there and the second top is far away from Gaussian so therefore another experiment was done to get a better estimation. The reason that the top is far away from Gaussian can depend on several different things but my guess is that it was because of an air bubble.
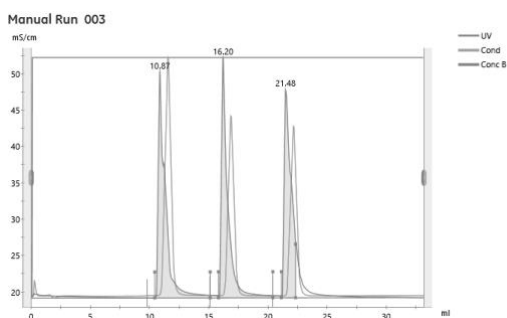


*Figure D.1. Epsilon estimation with the column showing the retention time for the UV sensor.*
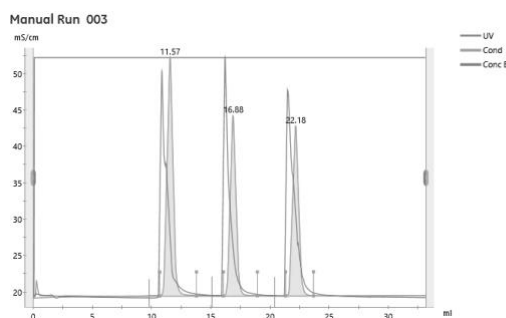


*Figure D.2. Epsilon estimation with the column showing the retention time for the conductivity sensor.*
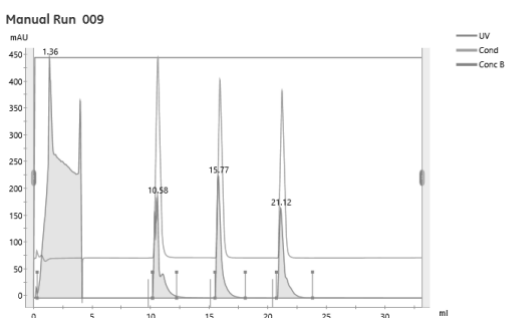


*Figure D.3. Epsilon estimation without the column showing the retention time for the UV sensor. Only the two last peaks has been used.*
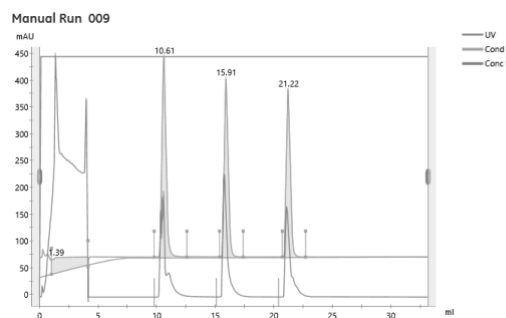


*Figure D.4. Epsilon estimation without the column showing the retention time for the conductivity sensor. Only the two last peaks has been used.*
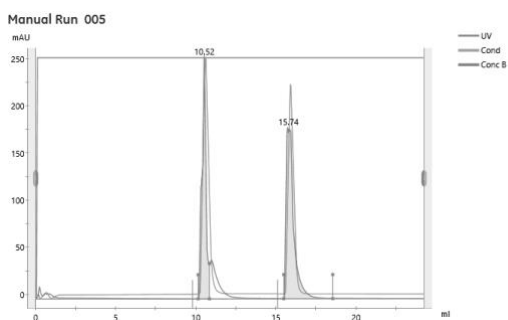
*Figure D.5. Epsilon estimation without the column showing the retention time for the UV sensor. Only the first peak has been used.*
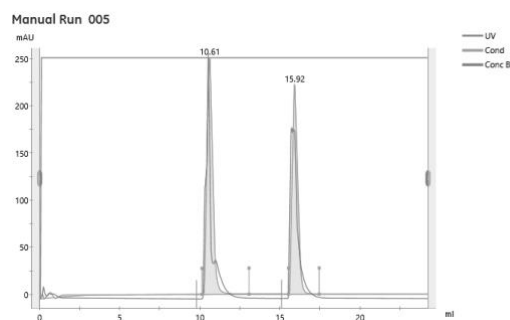
*Figure D.6. Epsilon estimation without the column showing the retention time for the conductivity sensor. Only the first peak has been used.*

*Table D.1. Shows the injection time, the retention time for all the peaks with the column and the difference between the detection time and injection time in figure D.1 and D.2.*

| Peak | Injection time (ml) | UV detection time (ml) | Conductivity detection time (ml) |
|---|---|---|---|
| 1 | 9.8 | 10.87 | 11.57 |
| 2 | 15.11 | 16.20 | 16.88 |
| 3 | 20.41 | 21.48 | 22.18 |

| Peak | UV time − injection time (ml) | Conductivity time − injection time (ml) | |
|---|---|---|---|
| 1 | 1.07 | 1.77 | |
| 2 | 1.09 | 1.77 | |
| 3 | 1.09 | 1.77 | |
| Mean value | 1.077 | 1.77 | |

*Table D.2. Shows the injection time, the retention time for all the peaks without the column and the difference between the detection time and injection time. Peak 1 comes from figure D.5 and D.6 and peak 2 and 3 comes from D.3 and .D4.*

| Peak | Injection time (ml) | UV detection time (ml) | Conductivity detection time (ml) |
|---|---|---|---|
| 1 | 9.8 | 10.52 | 10.61 |
| 2 | 15.11 | 15.57 | 15.91 |
| 3 | 20.41 | 21.12 | 21.22 |

| Peak | UV time – injection time (ml) | Conductivity time – injection time (ml) |
|---|---|---|
| 1 | 0.72 | 0.81 |
| 2 | 0.66 | 0.80 |
| 3 | 0.71 | 0.81 |
| Mean value | 0.697 | 0.807 |

With the help of table D.1 and D.2 the time for blue dextran and sodium chloride to pass through the column can be seen in table D.3.

*Table D.3. Values from table D.1 and D.2 used to calculate Δt.*

| Variable | Time | Variable | Time |
|---|---|---|---|
| UV – injection with column (ml) | 1.077 | Cond – injection with column (ml) | 1.770 |
| UV – injection without column (ml) | 0.697 | Cond – injection without column (ml) | 0.807 |
| $\Delta t_{dextran}$ | 0.380 | $\Delta t_{NaCl}$ | 0.963 |

With the result from table D.3 and equations 3.3, 3.4 and 3.5 the epsilons was calculated and is shown in table D.4.

*Table D.4. Shows the three different calculated epsilons.*

| Parameter | Value |
|-----------|-------|
| $\varepsilon_c$ | 0.380 |
| $\varepsilon_p$ | 0.940 |
| $\varepsilon$ | 0.963 |

## 8.5 Appendix E: Sensitivity Analysis

This Appendix shows the sensitivity analysis of all the parameters. The analysis was done by changing the parameters by multiplying with ten and differentiates with ten one at the time. As can be seen in figures E.1-E.8 the changes along the x axis is none or very small for $k_{max}$, $S_1$, $S_2$ and $k_{film}$. For $k_a$ and n the changes are significant and therefor it was chosen to calibrate on these two as can be seen in figures E.8-E.12. The starting values in this sensitivity analysis can be seen in table4.2 and 4.4.
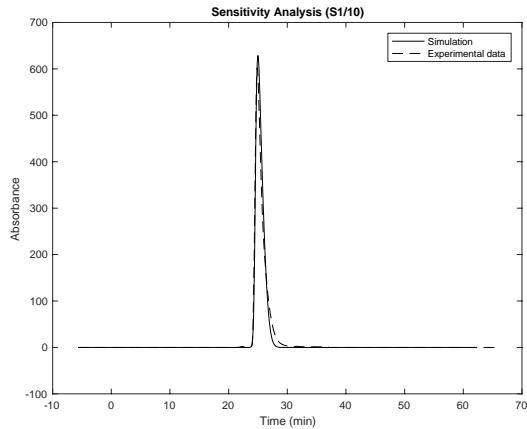


*Figure E.1. Sensitivity analysis with S1 differentiated by 10.*
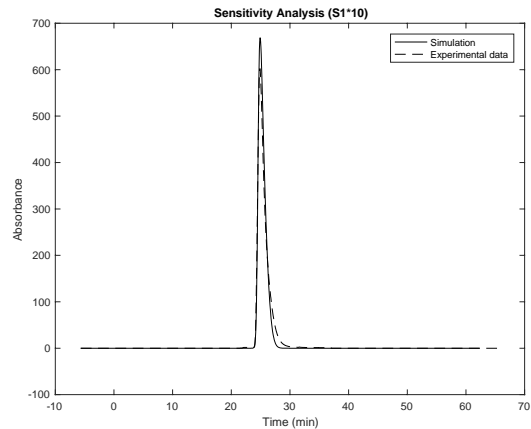


*Figure E.2. Sensitivity analysis with S2 multiplied by 10.*
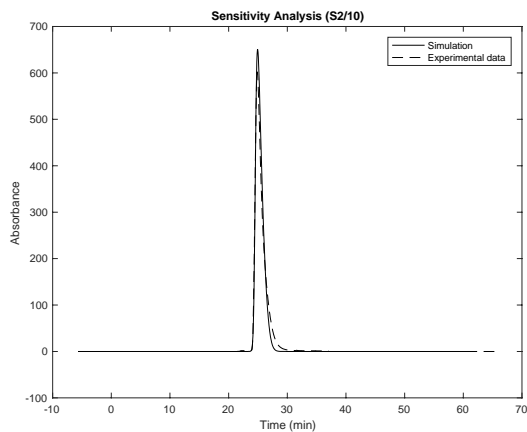


*Figure E.3. Sensitivity analysis with S2 differentiated by 10.*
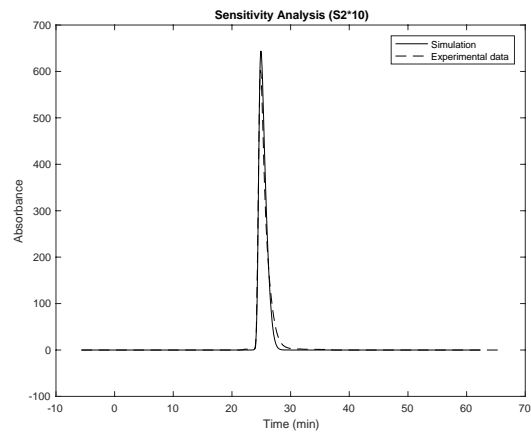


*Figure E.4. Sensitivity analysis with S2 multiplied by 10.*
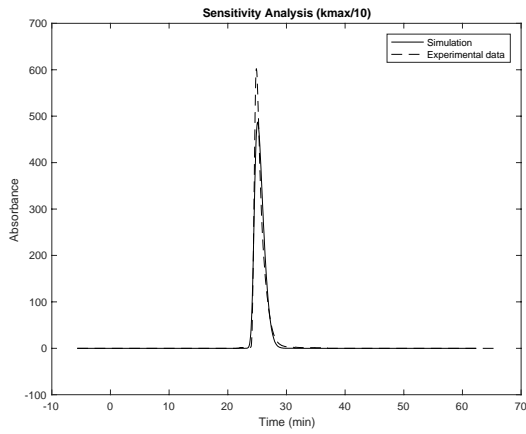
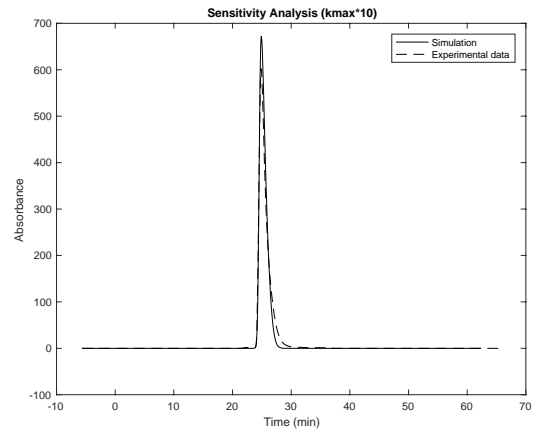*Figure E.5. Sensitivity analysis with $k_{max}$ differentiated by 10.*



*Figure E.6. Sensitivity analysis with $k_{max}$ multiplied by 10.*
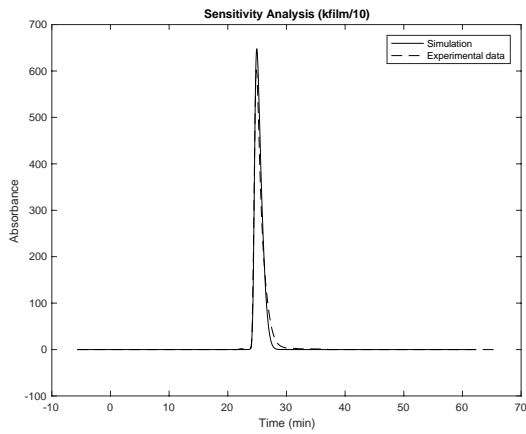


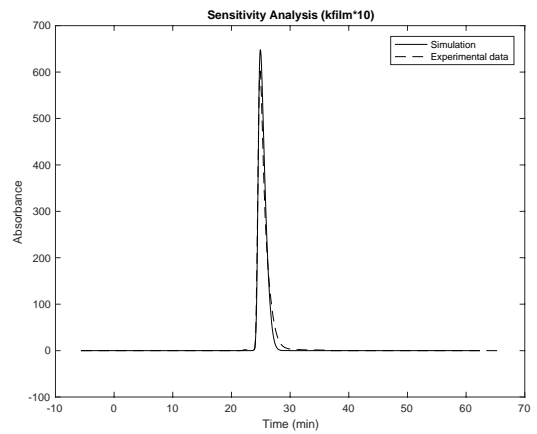*Figure E.7. Sensitivity analysis with $k_{film}$ differentiated by 10.*



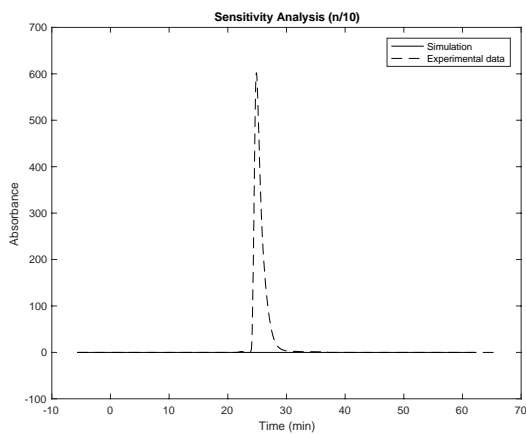*Figure F.8. Sensitivity analysis with $k_{film}$ multiplied by 10.*



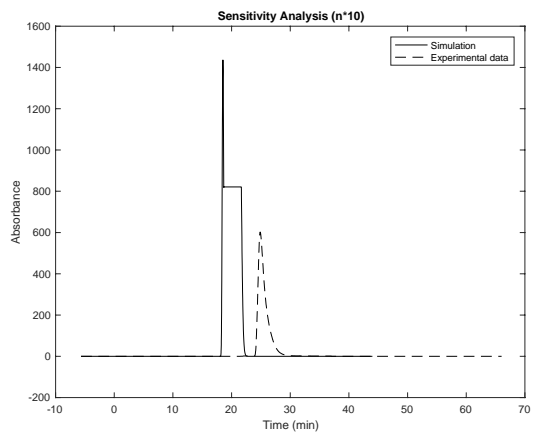*Figure E.9. Sensitivity analysis with n differentiated by 10.*



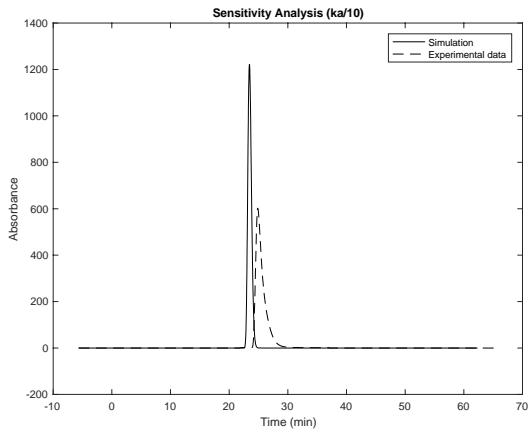*Figure E.10. Sensitivity analysis with n multiplied by 10.*

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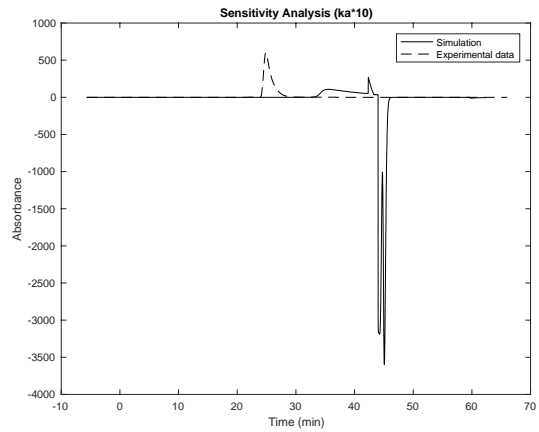*Figure E.11. Sensitivity analysis with ka differentiated by 10.*

*Figure E.12. Sensitivity analysis with $k_a$ multiplied by 10.*

An calibration for the same experiments as shown in section 4.2.2 Parameters Determined without Gradient was made but this time with all six parameters. The result of this calibration can be seen in figure F13 and the parameters in table F1.
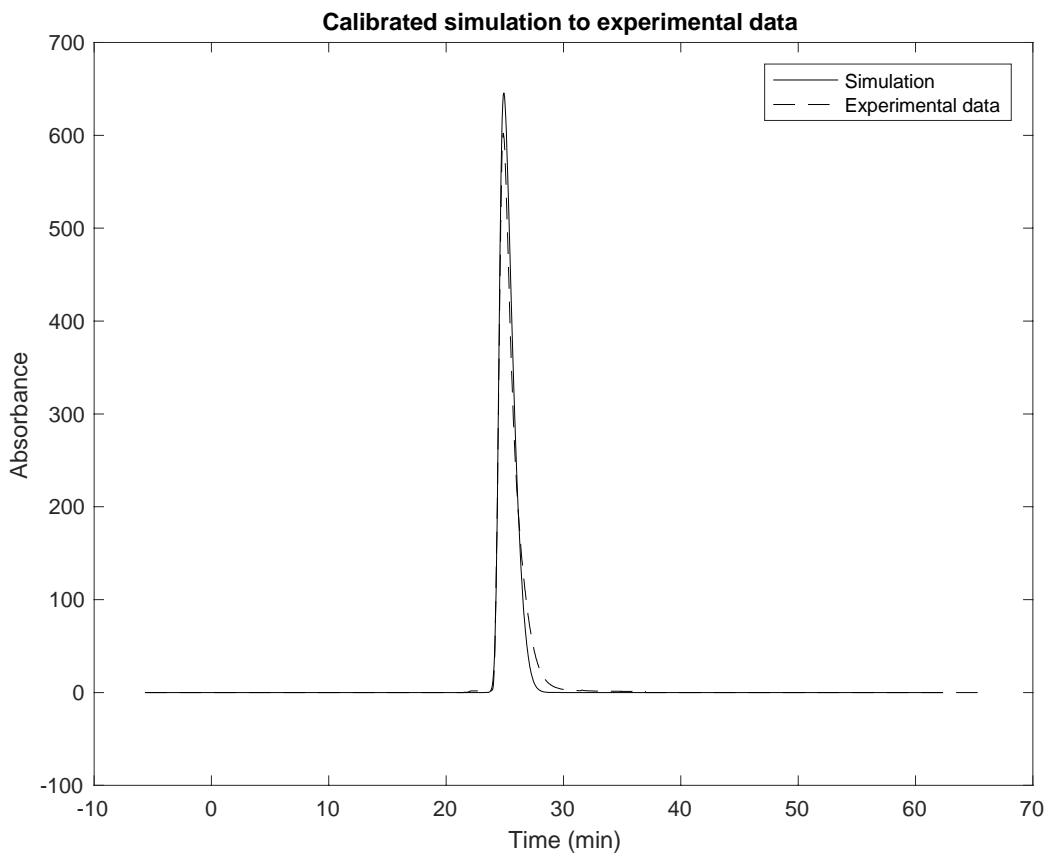


*Figure E.13. Calibration of all six parameters. Same experimental data as in section 4.2.2 Parameters Determined without Gradient.*

*Table E.1. Calibrated parameters for figure E.13*

| Parameter | Value |
|---|---|
| $k_{max}$ | $1.32 \cdot 10^2$ |
| $S_1$ | 0.490 |
| $S_2$ | 4.33 |
| $K_a$ | $5.47 \cdot 10^5$ |
| $n$ | 11.7 |
| $k_{film}$ | $9.24 \cdot 10^4$ |

Since the difference between figure 4.2 and figure E.13 are small, the values of n and $K_a$ in table E.1and table 4.4 are similar and with the help of the sensitivity analysis it was decided that calibration on $K_a$ and n gave a satisfying result. To only calibrate on two parameters also decreased the calibration time from 2300 seconds to 133 seconds which is about 6% of the simulation time.

## 8.6 Appendix F: Popular Abstract (Swedish)

När läkemedel produceras bildas även en massa biprodukter. Dessa biprodukterna kan många gånger vara mycket skadligare än vad själva läkemedlet gör nytta. Därför renar man upp läkemedel med en process som heter kromatografi. Kromatografi är som ett rör som är fyllt med något typ av sand eller sten. För att sedan rena upp sitt läkemedel låter man sin blandning med produkt och biprodukt åka genom kolonen och separeras. Man får då alla ämnen var för sig.

Under detta examensarbete har en särskild sorts kromatografi undersökts som heter affinitetskromatografi. Affinitetskromatografi fungerar som så att det enbart riktar in sig på ett förutbestämt ämne som det fångar upp och släpper genom resten. Låt säga att man har sin kolon fast denna gången har den packats med en massa små magneter. Därefter låter man en blandning av järnspån, träspån och sand åka genom. När detta passerar genom kolonnen kommer enbart järnspånet att fastna på magneterna och resten kommer bara att passera genom. Därefter låter man en vätska åka genom kolonen som avmagnetiserar magneterna för att spola ut järnspånet och sen en ny vätska för att göra magneterna magnetiska igen så man kan upprepa processen många gånger.

I detta examensarbetet undersöktes även utflödesprofilen då kolonen fylldes på. Detta eftersom det är önskat att fylla kolonen så mycket som möjligt utan att det som man vill fånga upp börjar rinna genom. Det kan ses lite som att man har två tunnor och vatten i den ena som man vill flytta till den andra. Skulle man då bara fylla lite grann i spannen och gå med eller skulle man fylla så mycket som möjligt i spannen för att behöva gå färre gånger? För de flesta skulle svaret vara att fylla spannen så mycket som möjligt varje gång, men när man fyller spannen helt är det också väldigt lätt att det rinner över innan man hinner stänga kranen. Av denna anledningen har det undersökts hur mycket rinner över ur spannen när man fyller den om man inte öppnar kranen fullt varje gång och hur påverkar spannens storlek detta?

Som en följd på att fylla spannen undersöktes även CaptureSMB. Det är när man försöker att fylla spannen utan att stänga av kranen. I detta fallet håller man den ena spannen över den andra medan man fyller för att fånga upp det som rinner över. När första spannen är full går man och tömmer den och sen när man kommer tillbaka sätter man den tomma spannen under den andra så att den fångar upp när den rinner över. På detta sättet behöver aldrig kranen stängas av vilket gör processen effektivare. I en del processer är det inte heller möjligt att stänga av kranen och då blir detta extra viktigt.

Texten ovan är en populärvetenskaplig sammanfattning av examensarbetet *Modeling &Simulation of Affinity Chromatography & Investigation of CaptureSMB* av Dennis Bogren