SMALL SCALE MONOCLONAL ANTIBODY PURIFICATION PLATFORM

by

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Preface

This report is the result of my master thesis at the department of chemical engineering. I would like to express my gratitude to professor Bernt Nilsson for giving me this opportunity to work with both Orbit and the ÄKTA pure machine. I also much appreciated the support given and brainstorming sessions together.

I am grateful for all the support my supervisor Niklas Andersson provided to help me out with Orbit and Python, especially with complicated troubleshooting and the parallel implementation.

I owe a big thank you to my co-supervisor Peter Tiainen for giving insight in industry practice regarding monoclonal antibody purification processes and I enjoyed our brainstorming sessions as well.

And an additional thank you to Joaquin Gomis Fons for helping me out with the laboratory work.

Abstract

The aim of this project is to transfer and implement a monoclonal antibody purification process that is traditionally done on an ÄKTAxpress to another machine called the ÄKTA pure. This is due to the fact that the ÄKTAxpress is being discontinued by the manufacturer and a replacement machine is needed for the time being. The project will include developing some new methods that might also highlight the industry's need for more advanced methods when an eventual replacement machine will reach the market.

The purification process consists of firstly affinity chromatography followed by size exclusion chromatography. The implementation of this purification process was done with the help of external Python-based control system Orbit.

The implementation went well and the chromatograms produced showed clearly the load, wash and elution stages of the respective columns. Three pumps were used to load and wash the serially connected columns. Some new methods that were created using Orbit was concentration-based pooling and sample change depending on sample volume. While testing the system during longer runs, the process yields were constant and therefore the system was considered quite robust.

Another proof of concept implementation of the purification process was developed that used parallel flow paths with the aim to shorten overall process time. Instead of using three pumps to load and wash all columns, two pumps were connected to the affinity column and one to the size exclusion column. Using a special kind of valve, these two flow paths were connected to allow loading the size exclusion column from the eluate of the affinity column. This did indeed increase the process productivity by roughly 30%.

The ÄKTA pure has a larger sample loading capacity per machine than the ÄKTAxpress. In addition, it also has a larger fraction collector. With the possibility to run the process both serially and in parallel, it is concluded that the ÄKTA pure is more than capable to replace the ÄKTAxpress.

Sammanfattning

Syftet med detta projekt var att överföra och implementera en monoklonal antikroppsupp-reningsprocess som oftast utförs av kromatografisystemet ÄKTAxpress på systemet som kallas ÄKTA pure. ÄKTAxpress kommer inte längre att stödjas av tillverkaren vilket ger behov av identifera, testa och överföra nuvarande process till temporärt ersättningssystem. Vidare leder implementering av process i ny systemmiljö till att nya metoder utvecklas och som kan belysa industrins framtida behov och eventuellt tillgängliggöras och implementeras i framtida mark-nadslösningar.

Uppreningsprocessen utgörs av två kromatografisteg, det första steget är affinitetskromatografi och därefter följer gelfiltrering. För att kunna utföra implementeringen av uppreningsprocessen i detta projektet användes ett externt Python baserat styrsystem som kallas Orbit.

Implementeringen var framgångsrik och alla lyckade kromatogram visade tydliga laddnings-, eluerings- och tvättningsfaser. Tre olika pumpar användes för att ladda och tvätta de två olika kolonnerna som var seriellt kopplade. Några av de nya skapade metoderna i Orbit var koncent-rationsbaserad pooling samt byte av prover beroende på provernas volym. Då systemet testades under längre tidsperioder visade det sig att produktsutbyten var ganska konstanta och därmed kunde man dra slutsatsen att systemet var robust.

Ytterligare en konceptsimplementering utvecklades där man använde sig av två parallella flö-desbanor i syfte att korta ner processtiden. Istället för att använda sig av tre pumpar till båda kolonner, kunde man istället fördela två pumpar till affinitetskolonnen och en pump till gelfil-treringskolonnen. Med hjälp av en särskild ventil kunde man koppla ihop flödesbanorna med varandra så att laddning av gelfiltreringskolonnen från affinitetskolonnen möjliggjordes. Re-sultatet blev att produktiviteten ökade med ungefär 30%.

ÄKTA pure har möjlighet att ladda fler prover per maskin samt har även större kapacitet i sin fraktionsuppsamlare än ÄKTAxpress. Dessutom, möjligheten att köra processen både seriellt och parallellt gör att ÄKTA pure kan bli en utmärkt ersättare till ÄKTAxpress.

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1 Introduction

As of winter 2022, the world is still haunted by the Covid-19 virus with its devasting effects and the words “vaccine” and “antibody” is on everybody’s lips. The antibody, which is this small protein that aids the immune system to locate and eliminate foreign substances, is vital for the fight against deadly diseases [1]. The research and production of antibodies is an ever-growing industry within the biopharmaceutical sector and they are used for diagnostic, analytic and therapeutic purposes [1,2]. A subgroup of antibodies, monoclonal antibodies, which are antibodies that originates from a single white blood cell, is produced in-vitro [1]. This certainly costs more, but monoclonal antibodies are highly sensitive [1]. Before these monoclonal antibodies ever reach the wide public market, they have to be evaluated and tested. This means that a large number of different variants of monoclonal antibodies are being produced for this preparative scale and for this reason reliable, fast and cheap purification processes are needed [2]. The most common way of purifying monoclonal antibodies is using chromatography [2]. Chromatography is a separation process where substances are separated from a mixture due to their chemical and physical characteristics.

There are several chromatography systems available on the market, one of them being the ÄKTA-series made by Cytiva. The chromatography system called ÄKTAxpress is designed for preparative recombinant protein purification, which includes the purification of monoclonal antibodies. It’s an automated system that allows for two-step liquid chromatography and the system is widely used in the biopharmaceutical industry. However, Cytiva has actually discontinued the ÄKTAxpress, which will take in effect 2023 [3]. As the system will be no longer supported, the need for a replacement system is of utmost importance.

Fortunately, another ÄKTA product, the ÄKTA pure, could potentially fill the void left by the discontinued ÄKTAxpress. The ÄKTA pure is a small scale, highly flexible and customisable liquid chromatography system. In addition, it is not limited to its default operating program, meaning external control systems can be used.

Orbit is a Python-based chromatographic control system developed by the department of chemical engineering at Lund university. Using Orbit to control the ÄKTA pure, new advanced methods can be created and will highlight some of industry’s needs to be considered when developing a new system.

1.1 Aim

The aim of this master thesis is to transfer and implement the monoclonal antibody purification process done by a ÄKTAxpress to a ÄKTA pure using the python-based control system Orbit and develop some methods that highlight the industry’s needs. Moreover, a parallel implementation of the purification process using two flow paths will also be developed and compared to the regular serial implementation.

2 Background

2.1 Monoclonal antibody purification

The industrial downstream process for purifying and recovering monoclonal antibodies is relatively straight forward. Cells secret their produced monoclonal antibodies into the surrounding batch medium. The batch is later centrifuged and the supernatant is harvested. The supernatant is run through two purification steps, Protein A chromatography and some chromatographic polishing steps. Some viral inactivation steps are included here as well. Lastly ultrafiltration or diafiltration is used as a final polishing step. [2]

On a preparative scale, some of the steps mentioned above aren't needed as the product will never reach the market, but instead used for further research and evalution. The most common way of monoclonal antibody purification is then firstly to run the supernatant through an affinity chromatography step, and later, one or more de-salting steps which could be ion exchange chromatography or size exclusion chromatography [4]. In this project, the preparative scale is in focus and therefore the first step will be Protein A chromatography followed by size exclusion chromatography.

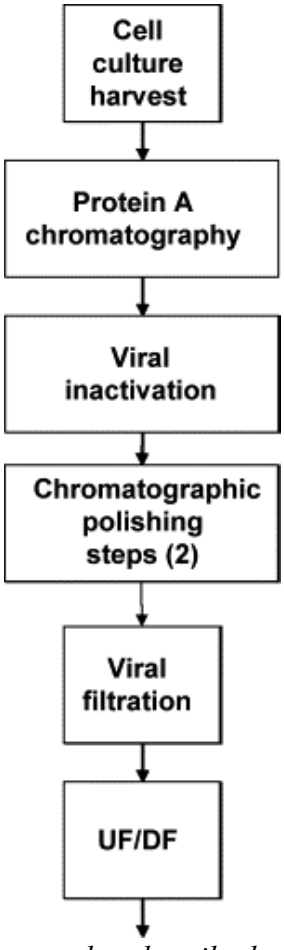

*Figure 2.1. Downstream process of monoclonal antibody productio*n. [2]

2.1.1 Protein A Chromatography

Protein A chromatography is a type of affinity chromatography that is able to achieve high purity and yield for monoclonal antibodies [4]. Protein A originates as a membrane protein from *Staphylococcus aureus* and it has a strong affinity for the Fc region of especially IgG [5]. This makes it an ideal capture ligand as most other biochemical components that exist in the cultivation liquid such as other proteins and DNA will simply pass through the column [4]. It also has the ability to bind to certain variants of Fab-fragments making it more versatile in the world of monoclonal antibody production and development [5].

In the column, Protein A is coupled with a resin that allows one Protein A ligand to bind two Fc-regions [5]. The resin usually has lifespan of 200 cycles, but it depends on the type of resin, the amount of Protein A that is leached with each cycle and chemical degradation made by the cleaning procedures [4]. On the other hand, the cleaning procedure of the column usually is quite simple and fast.

2.1.2 Size Exclusion Chromatography (SEC)

Size exclusion chromatography separates molecules depending on their size. Large molecules travel faster through the SEC column as it is packed with beads with varying porosity. The smaller molecules take longer time as they can get trapped in the beads' pores [6]. Together with a more selective and sensitive chromatography step applied before, the SEC works well as a polishing step to remove salts and but most importantly, to separate the aggregates and fragments from the monomer antibodies [6]. However, in many monoclonal antibody purification processes, the wash and cleaning steps of the column is a bottleneck due to its time-consuming procedure [6].

The size exclusion chromatography column will from now on be referred as simply SEC.

2.2 ÄKTAxpress

ÄKTAxpress is a chromatography system that allows for automated multistep purification. It is a system that works well for established ways of purifying recombinant proteins and in this case, it is being used for monoclonal antibody purification. The machine itself has a rather low footprint and several ÄKTAxpress can be stacked together horizontally to increase the productivity. The default control system is called Unicorn. [7]

The ÄKTAxpress system that is to be implemented on a ÄKTA pure uses two step purification with the columns set up as explained above in 2.1, Protein A chromatography followed by SEC. The liquid flow paths of the ÄKTAxpress can be seen in figure 2.2 which also includes all the available modules and connections. Starting from the bottom of the figure, it can be seen that the inlet valve handles both the buffers and the samples. Up to four samples can be used and 10 buffer inlets [7]. Afterwards, the liquid flows through the system pump up to the injection valve. The injection valve can handle manual injection and has connections to the loop valve and column valve respectively. The loop valve is able to connect to five loops [7]. The column valve can be connected up to 5 columns depending on their size, and from here, the liquid continues flowing through a UV-sensor, a conductivity meter and a flow restrictor and ends up at the outlet valve [7]. Here it can be sent to waste, stored in flasks or stored in one integrated 96-well plate [7]. This plate will be filled up in roughly 2 days of continuous use.

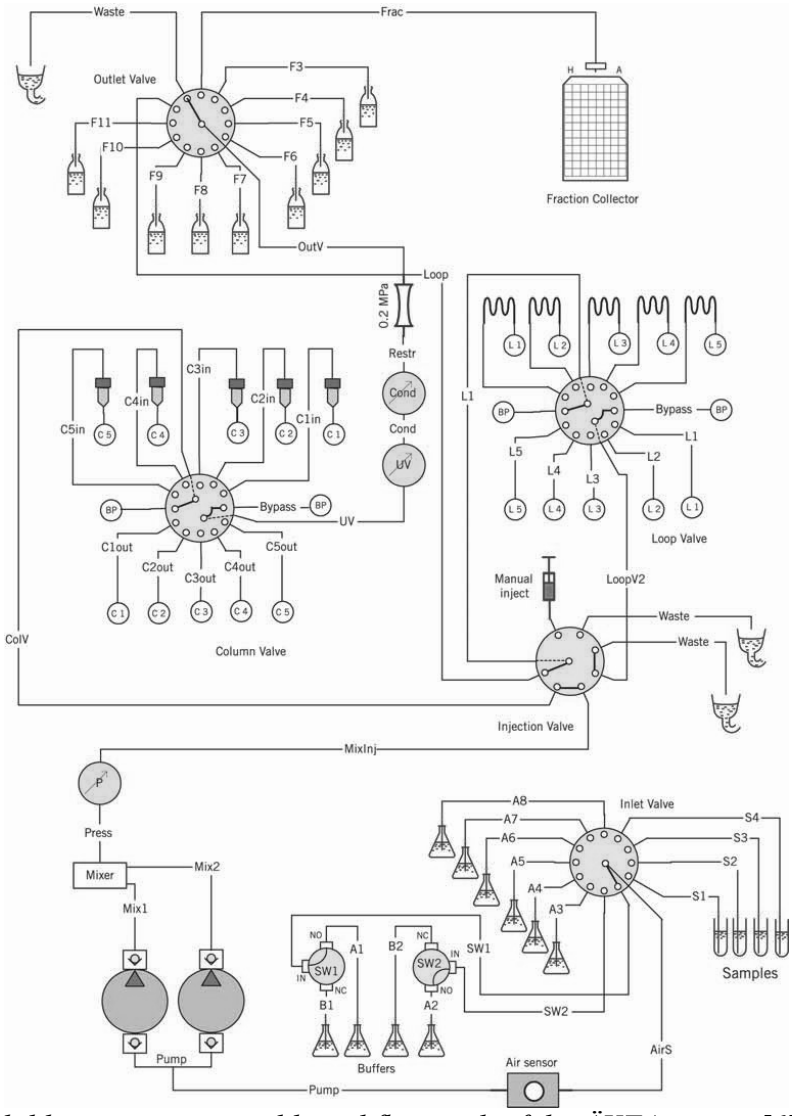

Figure 2.2. Available connections and liquid flow path of the ÄKTAxpress. [6]

2.3 ÄKTA pure

ÄKTA pure is a highly flexible chromatography system that allows the user to configure the process and system to a high extent so it can run both simple and advanced purification processes. The default control system is Unicorn, but the ÄKTA pure supports external control systems such as Orbit which will be used throughout this project. Additional modules can be added to expand the system. The system is three times more expensive and three times larger than the ÄKTAxpress, but in contrast it can utilize two different system pumps with an additional sample pump. [8]

A typical example of the liquid flow path is shown in figure 2.3. As there are two system pumps on an ÄKTA pure, each system pump can have its own inlet valve which each has 7 inlet ports. There is an additional inlet valve IX that can be connected which has 8 inlet ports. The sample pump also comes with its own sample inlet valve with 7 inlet ports. The column and loop valves have 5 ports each. Unicorn allows up to 4 so called versatile valves which are valves that can be added to create extra features. Moreover, ÄKTA pure can be connected to a fractional collector that can contain up to six 96-well plates. [8]

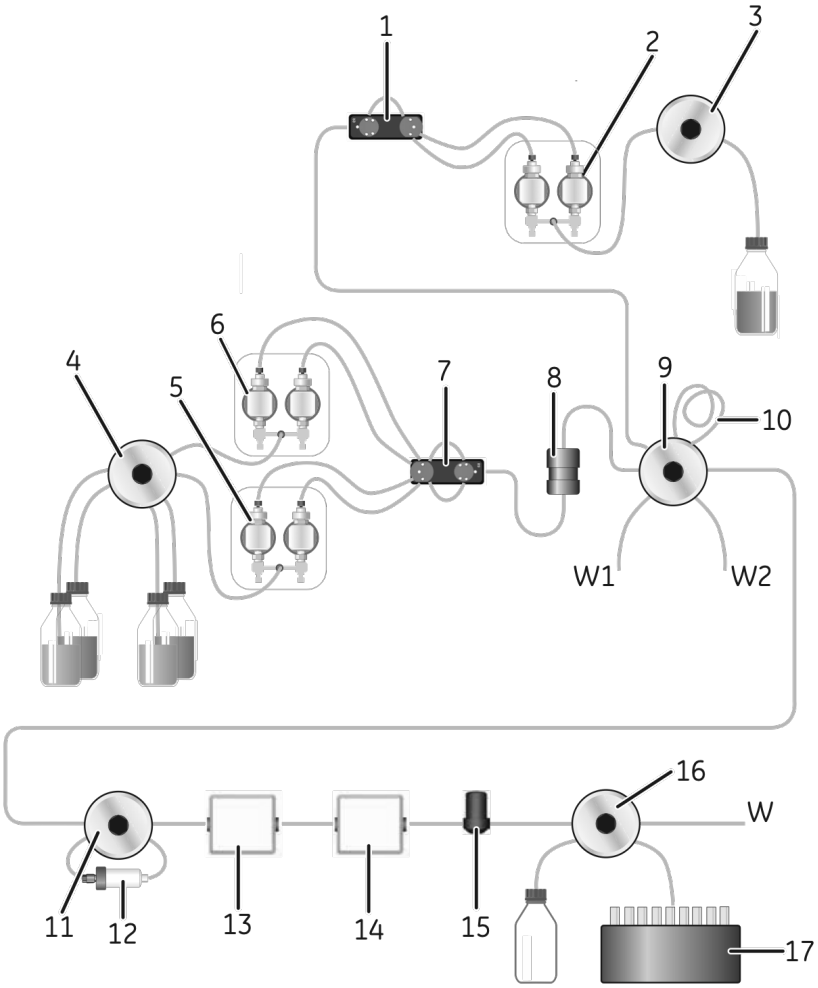

Figure 2.3. Illustration of a typical liquid flow path of the ÄKTA pure.Components in numeric order as shown in figure: 1 – Pressure monitor, 2 - Sample pump, 3 – Sample inlet valve, 4 – Inlet valve, 5 – System pump B, 6 – System pump A, 7 – Pressure monitor, 8 – Mixer, 9 – Injection Valve, 10 – Sample loop or superloop, 11 – Column valve, 12 – Column, 13 – UV monitor, 14 – Conductivity meter, 15 – Flow restrictor, 16 – Outlet valve, 17 – Fraction collector, W,W1,W2 – Waste. [8]

2.4 Orbit

Orbit is a Python based chromatography control system that was developed at the department of chemical engineering at Lund University [9]. It was built using the same methods of Unicorn, but also includes more advanced methods and customisation since it's built in Python [8]. It works by executing commands in orbit, which are then sent to a Unicorn server that connects with the ÄKTA machine being used [9].

Some great advantages of using orbit as the controller instead of Unicorn is the possibility to run parallel flow paths and allows the user to customise the system and operations beyond the limits set by Unicorn [9].

When using orbit, three separate scripts are needed, these being the system, process and run scripts.

In the system script the user defines all the units (valves, pumps etc) that are going to be used. The units are selected from a vast library that mirrors the available modules for the ÄKTA pure and each unit comes with some pre-defined methods. Default values and maps can be created here within each unit. The flow path is also defined here, using the tube class to connect two units with each other. Later a system class is defined that contains all the units. [9]

In the process script all the methods that are going to be used in the actual process is defined such as flowrate, gradients and pooling. Most of these methods are used when defining the different phases that is going to take place, but some methods can be used as events. Events are instructions that require conditions to be fulfilled and are suitable for more complicated tasks such as pooling. [9]

The run script is where the user finally defines the phases that are going to be executed. A phase contains different methods that has to be defined before in the process script. An example of a phase can be seen below in figure 2.4.1.

```
Flush = Phase(name = 'Flush',
              SetInlet = {'%B': 0, 'A':4},
              Valve = {'column': 0, 'inject': 'Manual load', 'loop': 5},
              FlowRate = 1,
              Time = 24*60)
```

Figure 2.4.1.: An example of a phase.

This phase is called "Flush". The "SetInlet" method sets the concentration of B and which position the inlet valve is going to use. In this case, the concentration of B is 0% and the inlet valve A uses port number 4. It is thus a purely buffer A defined phase. The "Valve" method sets the called upon valves to a desired position. In this case the column valve is bypassed (position 0), the injection valve is set to manual load and the loop valve is set to position 5. The "FlowRate" method sets the system flowrate and the "time" method sets the duration of the phase.

When all the phases have been defined, the phases have to be compiled into a ordered list. Orbit will read and execute the phases in the order they are placed in the list. It is possible to tinker with this list to quite some extent and parallel phases can actually be implemented. More on this in section 3.2.

The process is started in the run script with two conditions required, the system class defined from the system script and the phase list. The process can be run in test mode to allow for troubleshooting and in real mode to connect to the ÄKTA pure and run the actual process. [9]

3 Materials & Methods

3.1 Serial implementation

The serial implementation used two serially connected chromatography steps. As mentioned in section 2.1, the affinity column comes first followed by a size exclusion column. The process diagram concept is shown below.

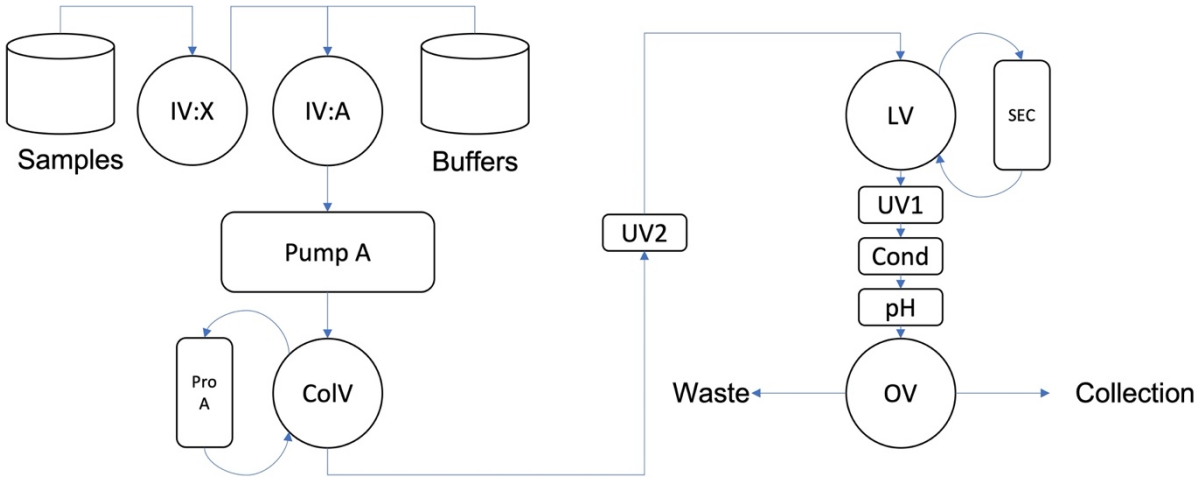

Figure 3.1.1. Process diagram concept. Blue lines represent available flow paths. It's a simple purification process consisting of two columns, firstly a Protein-A (Pro-A) affinity column and finally a size exclusion column (SEC). IV:X – Inlet valve X, IV: A – Inlet Valve A, ColV – Column valve, LV – Loop valve, OV- Outlet valve, UV1 – First UV sensor, UV2 – Second UV sensor, Cond – Conductivity meter, pH – pH meter.

In theory, the entire process could be run with only one pump as shown, but in practice both system pumps were used for buffers as it was easier and clearer to control since Orbit's base methods use two pumps. In addition, a separate sample pump was used for loading the affinity column to keep samples and buffers separate for the same reason as above, clearer overview. Therefore, the actual process implemented is shown below. Not shown in the figure was that each pump was connected to its respective inlet valve to allow for buffer selection.

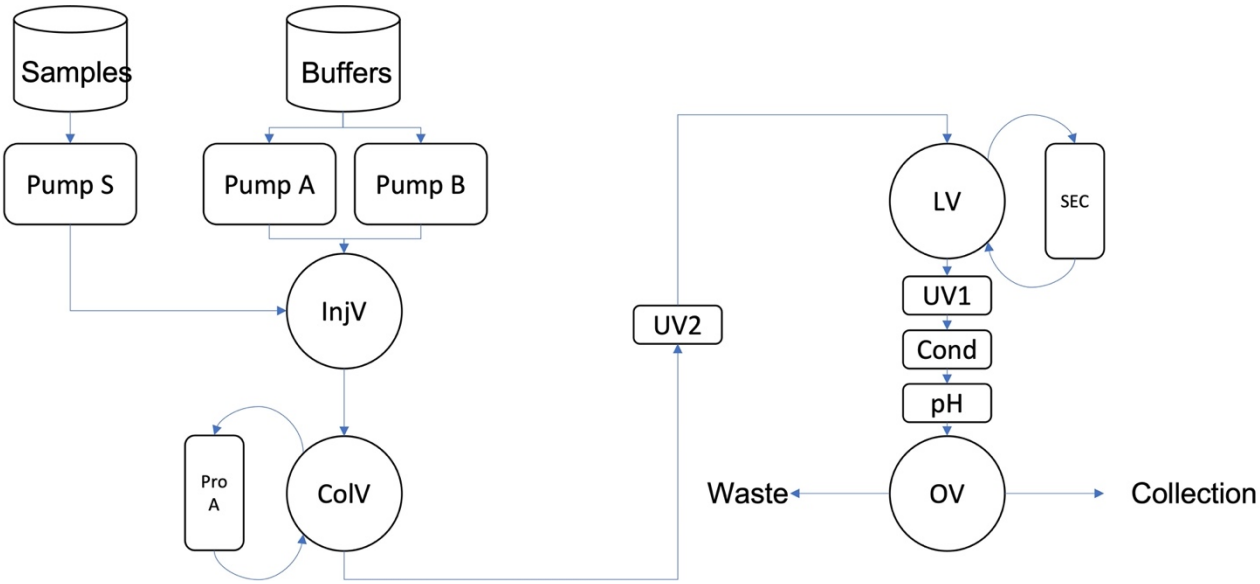

Figure 3.1.2. Real process diagram. Blue lines represent available flow paths. InjV – Injection Valve, ColV – Column valve, LV – Loop valve, OV- Outlet valve, UV1 – First UV sensor, UV2 – Second UV sensor, Cond – Conductivity meter, pH – pH meter.

The overall process is simple, but implementing the entire process at once would have been difficult, therefore the process was broken down into two parts, the affinity step and the SEC step. The affinity step was done first since the eluate of the affinity column would later be used as the load for the SEC. This is the reason why a second UV-sensor has to be used between the respective chromatography steps as it is required to pool the eluate.

In the table 3.1.1, an overview of the buffers and samples used for each phase is presented. The phases are also in the order they are executed in the phase list, with the affinity flush phase being the starting phase.

Table 3.1.1. The buffers and samples used for each phase. The phases are listed in the order of execution. Aff. – flow path through the affinity column, SEC- flow path through the size exclusion chromatography column. Buffer recipes can be found in table A.3.

Phase	**Buffer & Sample**
Flush (Aff.)	Deionized water
Flush (SEC)	Deionized water
Equilibrate (Aff.)	20mM Na-Phosphate 150mM NaCl pH 7.4
Equilibrate (SEC)	20mM Na-Phosphate 150mM NaCl pH 7.4
Load (Aff.)	Supernatant containing monoclonal IgG (concentration 0.8mg/ml)
Wash 1 (Aff.)	20mM Na-Phosphate 150mM NaCl pH 7.4
Wash 2 (Aff.)	50mM Na-Acetate pH 6
Elution (Aff.)	50mM Na-Acetate pH 3.5
Wash (SEC)	20mM Na-Phosphate 150mM NaCl pH 7.4 (was initially 50mM Na-Acetate pH 3.5)
CIP (Aff.)	0.1M NaOH
CIP (SEC)	1.0M NaOH
Flush (Aff.)	Deionized water
Flush (SEC)	Deionized water

3.1.1 Affinity column – Mabselect SuRe

The affinity column used was the 1ml Mabselect SuRe by Cytiva. The samples used throughout the project was a supernatant that contained monoclonal monomer IgG antibodies, the desired product to be purified. The concentration of the monomer antibody was 0.8mg/ml. It was likely that antibody fragments and aggregates were present in the supernatant as well.

Dynamic binding capacity is a measurement of the amount of antibodies that can be bound to the protein-A resin under specific flow conditions. The graph below shows the dynamic binding capacity at 10% breakthrough (DBC10) for the Mabselect SuRe and its newer counterpart Mabselect PrismA (which was not used). This graph was made by Joaquin Gomis Fons using DBC data from breakthrough curves with different flowrates [10]. A lower load of the SuRe was desired so to not waste sample volume and minimize operational time. Therefore, a residence time of 0.5 min was selected. The available DBC10 was thus 20mg/ml resin. A column void of 0.32 meant that the DBC10 per ml column was 13.6mg/ml column. The maximum load of the SuRe was 13.6mg of antibody since the column volume was 1ml. A load time of 15min was chosen, and the flowrate was set to 0.5ml/min, meaning a load volume of 7.5ml. Thus, the amount of antibodies loaded was 6mg. This in contrary to the maximum load of 13.6mg was in attempt to maximise yield and assume no loss of antibodies.

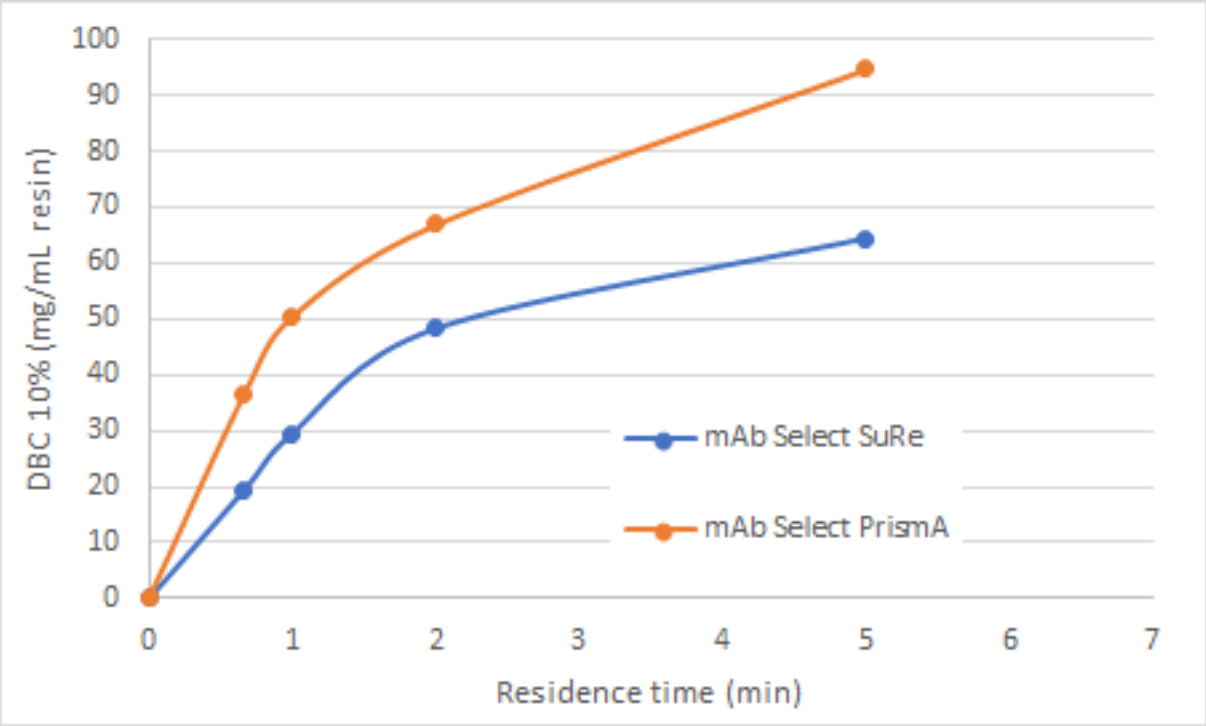

Figure 3.1. The dynamic binding capacity at 10% breakthrough of Mabselect SuRe and Mabselect PrismA. This graph was made by Joaquin Gomis Fons using dynamic binding capacity data from breakthrough curves with different flowrates. [10]

Once the load was decided, the pooling function had to be made. As mentioned before in section 2.4, an event was the best suited operation for this type of method as an UV signal would work as a trigger. The pooling event was scripted so that the user could define certain parameters; flowrate, UV start trigger, UV end trigger and in a later version even SEC load volume per cycle. The UV triggers are absorbance value so for example if the absorbance is greater than 1000mAU, an action would occur. And this action is the loading mechanism for the following

chromatography step, the SEC. When a absorbance value was reached, the loop valve would change position from bypass to the position the SEC was at and thus loading it. After the UV value was below the end trigger, the loop valve was set to bypass.

To get rid of tedious manual work and finding optimal end triggers, a SEC load volume parameter was added so only the start trigger was required and Orbit instead calculated the end trigger as a time condition using the set flowrate and SEC load volume. The start triggers for the UV was initially set to 1200mAU. The SEC load volume was initially set to 0.5ml.

3.1.2 SEC - Superdex 200 Increase

Once the pooling event functioned properly in test and real mode, the SEC could be loaded. The SEC used was a Superdex 200 Increase 10/300 GL from Cytiva. The volume of the SEC was 24ml. The SEC is quite sensitive to high flowrates and high back pressure and to mitigate this, the flowrates had to be set to 0.75ml/min for each phase that included the SEC instead of 1ml/min. This increased the overall operational time.

Initially, the wash SEC buffer used was the elution buffer used for the SuRe (pH 3.5). As mentioned before in section 3.1.1, the SEC load volume was 0.5ml which equalled a 2% load of the total SEC volume.

3.1.3 Single cycle

A cycle was defined as one iteration of the entire process i.e. going through the phases in table 3.1.1 once. A successful cycle would thus be a chromatogram that showed all the phases with their respective peaks. So, as the chromatography steps were working as they should, there was a need to optimize and change some parameter to more resemble industry practice. Therefore, the SEC load volume was increased from 0.5ml to 1.2ml, meaning a 5% load instead of 2%. The wash SEC buffer was also changed to the equilibration buffer (pH 7). Both these parameters have an effect on the peak from the SEC that will be discussed later.

The SuRe was loaded with 7.5ml of 0.8mg/ml of antibody supernatant, meaning a total of 6 mg of antibodies was loaded upon the SuRe as mentioned in section 3.1.1.

3.1.4 Multiple cycles

As a successful single cycle chromatogram was obtained, the long-term robustness of the system would now be tested. If the system could handle several consecutive cycles with similar yields for each cycle, the long-term testing would be considered successful.

To achieve this, some changes in the run script was made. A “cycles per sample” function was implemented that required some input to calculate the number of cycles the system would have to perform to process one sample flask (or more). The inputs were the volume of the sample flask, the desired minimal volume, the SuRe loading rate, the SuRe loading time and the number of sample flasks to be applied. The output was an appended phase list required by the system to run.

The function was first run in test mode to check that the system functions properly and later the function was run in real mode. The sample flask volume was set to 500ml, the minimal volume was 477.5ml, the SuRe loading rate 0.5ml/min and SuRe loading time 15min, meaning three cycles of a SuRe loading volume of 7.5ml was performed. Only one sample flask was applied in real mode.

3.1.5 Multiple cycles & various concentrations

Building upon the "cycles per sample" function described in the previous section, it was considered useful if the function could also handle different sample concentrations. This would allow the system to process multiple sample flasks with varied concentrations.

If the concentrations were to be changed, the pooling function in section 3.1.1 would have to be changed accordingly as well. Therefore, these functions were coupled in a way that the pooling function retrieved the current sample concentration and calculated the UV start trigger using the 0.8mg/ml as a relative reference. For example, the UV start trigger for 0.8mg/ml was set to 500mAU. If a sample with a concentration of 0.4mg/ml were to be applied, the UV start trigger would then be set to 250mAU.

In real mode, two tests were performed. The first test included two samples with the concentrations 0.4mg/ml and 0.8mg/ml and the second test included three samples with the concentrations 0.08mg/ml, 0.4mg/ml and 0.8mg/ml. The other input parameters were the same as the previous section (3 cycles per sample), meaning in the first test 6 cycles would be run, and in the second test 9 cycles would be run.

3.2 Parallel implementation

As in the serial implementation, this system also uses two chromatography steps. The units are essentially the same with the major difference being that there are two simultaneous parallel flow paths. One flow path that starts with pump A and takes care of the affinity column. The other one starts with pump B and takes care of the SEC. Unit wise, an additional valve had to be added, a versatile valve. The versatile valve allows for greater customisation and allows the affinity flow path to link up with the SEC flow path when needed. For the most part, the affinity flow path will be set to waste whilst pump B prepares the SEC.

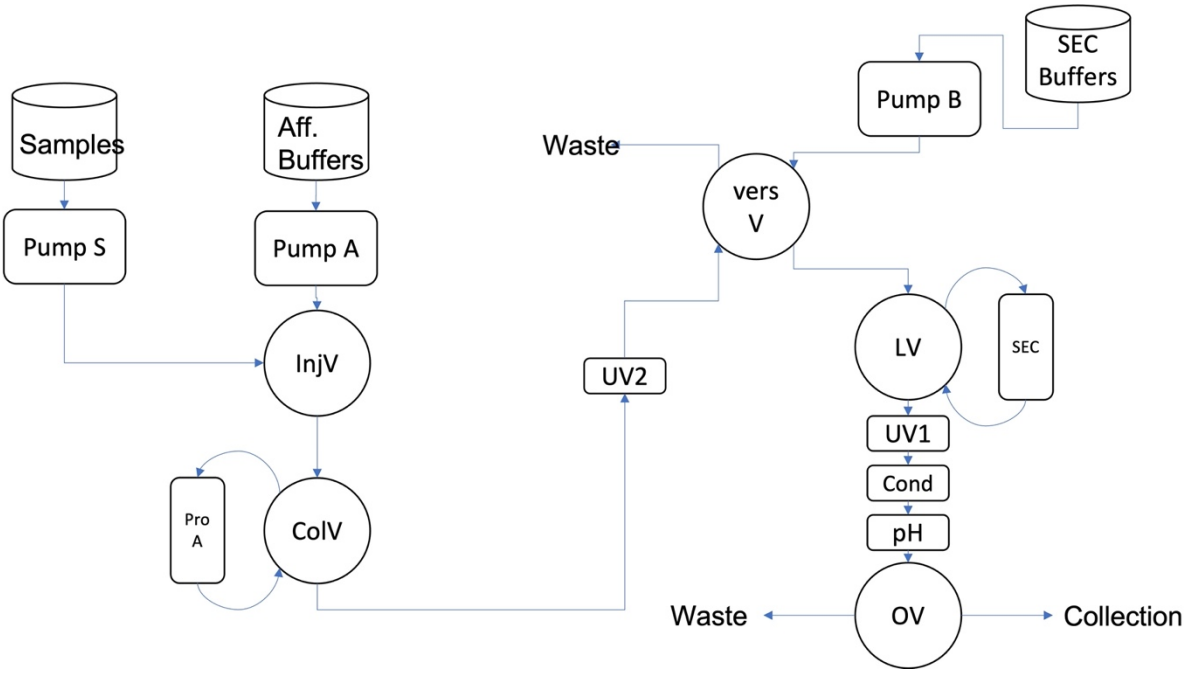

Figure 3.2.1. Parallel process diagram. Blue lines represent available flow paths. InjV – Injection Valve, ColV – Column valve, versV- versatile valve, LV – Loop valve, OV- Outlet valve, UV1 – First UV sensor, UV2 – Second UV sensor, Cond – Conductivity meter, pH – pH meter.

3.2.1 New methods and system

A few things had to be changed in the various scripts to make the above system work. Firstly, in the system script, the flow path had to be rewritten and a versatile valve had to be added. Secondly and most importantly, a method had to be created so the two system pumps could receive separate instructions. This is in stark contrast to how they are usually run since Unicorn requires a system flow rate (both pumps in total) and a percentage of how much pump B should be used. Now it was crucial that we could directly set a flow rate for both system pumps. After some tinkering the two methods were eventually made by using the current flow rates and the new flow rate to calculate the new total flow rate and the new percentage of B. The methods were defined under the pump unit and were called using the process script.

The pooling function also had to be improved since now not only the loop valve position had to be changed, the position of the versatile valve was equally important. This was easily done though. The default position of the versatile valve was that the affinity flow path goes to waste and the SEC flow path goes down to the loop valve. However, during the pooling event, the versatile valve would change position so the affinity flow path goes to the loop valve and the SEC flow path goes to waste and goes back to default setting once the event is over. An illustration can be seen below.

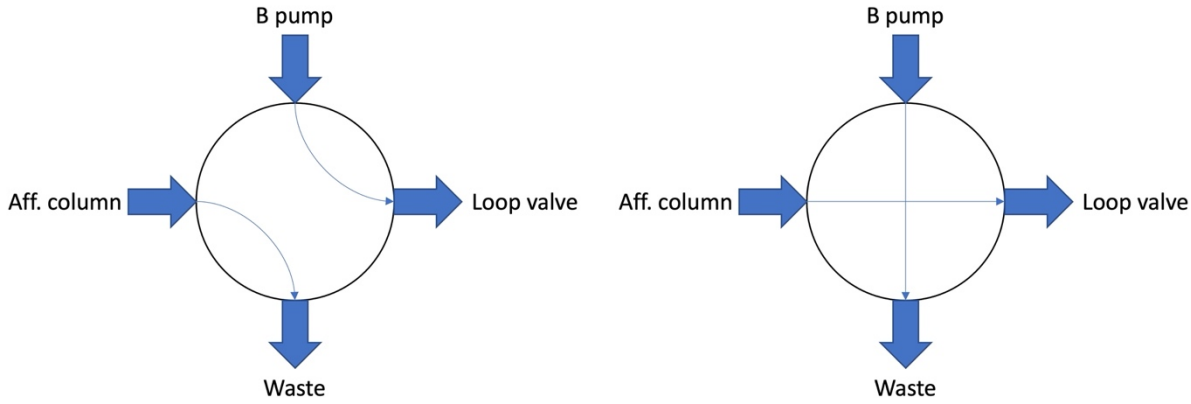

Figure 3.2.2. Illustration of the versatile valve and its default and pooling positions. Left: The default position. Right: The pooling position.

3.2.2 Single cycle

Once the test mode runs were successful it was time to reconfigure the ÄKTA pure system and change the tubing and valves to that of figure 3.2.1 so that the system could be run in real mode. As the process is run in parallel, most of the phases are run in different branches which are parallel lists of phases. An overview of the branched phase list can be seen below in figure 3.2.3. A wait phase had to be added to the affinity phases as these together are shorter than the two first preparative SEC phases. Once the equilibrate SEC phase is done, the elution phase merges the two branches together and initiates the pooling function. As the SEC has been loaded, the phases are once again branched to its respective wash and CIP phases. An additional wait phase has to be added to the end to synch up the branches and once the flush SEC phase is completed, the process ends.

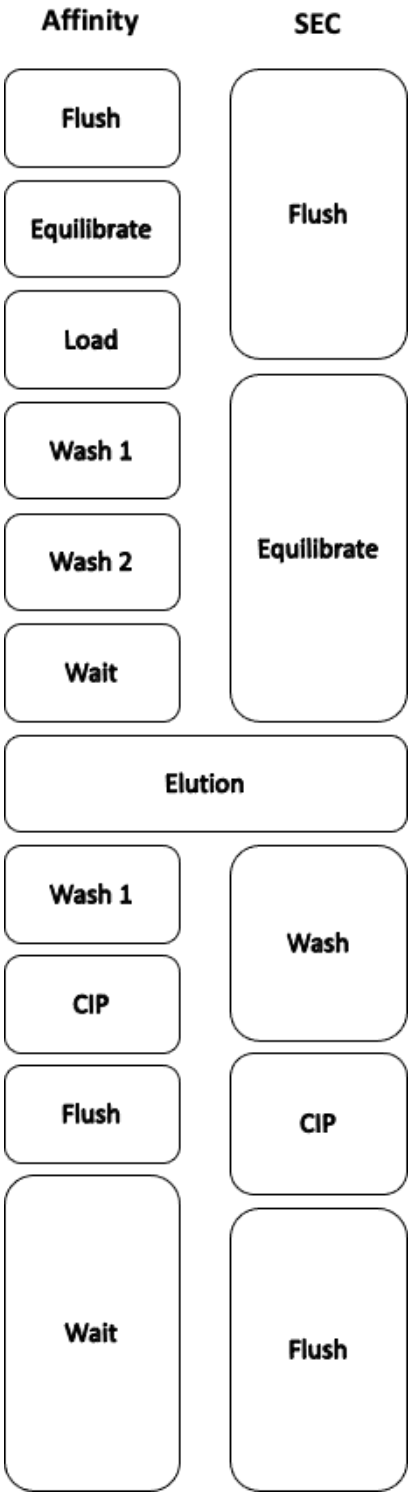

Figure 3.2.3. Overview of the branched phase list. The phases are not time scaled, but illustrates how some of the SEC phases are more time consuming.

All parameters were the same as in section 3.1.3 and only one cycle was run. The buffers were the same as in table 3.1.1.

3.2.3 Multiple cycles

The obvious next step was then to run the parallel process multiple times. No complicated function was implemented here as in section 3.1.4, instead a single "number of cycles" parameter was added before the phase list. The parameters were identical to that of the previous section, only difference was that the process ran the phase list 3 times. The most important result from this run was to see how much faster this process ran in comparison to that of the multiple serial run of section 3.1.4.

4 Results

To aid in comprehending the following chromatograms some label information is needed. The purple lines (and areas) are the absorbance values detected by the 2nd UV sensor (UV2), the one placed right after the Mabselect SuRe and before the SEC. The blue lines (and areas) are the absorbance values detected by the 1st UV sensor (UV1), the one placed after the SEC. These will be referred to as AC-UV sensor and SEC-UV sensor respectively throughout the section.

For a clearer overview of flow paths, check figures 3.1.2. and 3.2.1. in section 3.

4.1 Mabselect SuRe & Pooling function

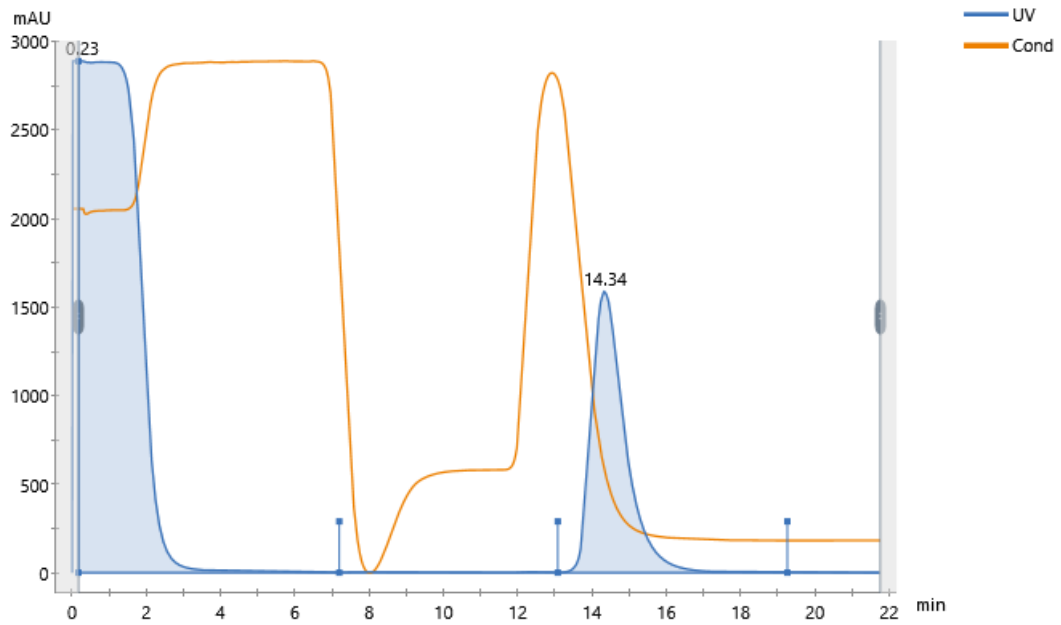

Figure 4.1.1. Elution of Mabselect SuRe. Left blue peak is the sample load, the second blue peak is the elution peak. UV (blue), conductivity (yellow).

In figure 4.1.1 a simple load and elute of the Mabselect SuRe is shown. No second UV sensor was installed here yet. The left large blue peak is the load peak and the second thinner blue peak is the elution peak. The elution peak is quite uniform, but gives little information regarding aggregates or fragments as there is only one peak.

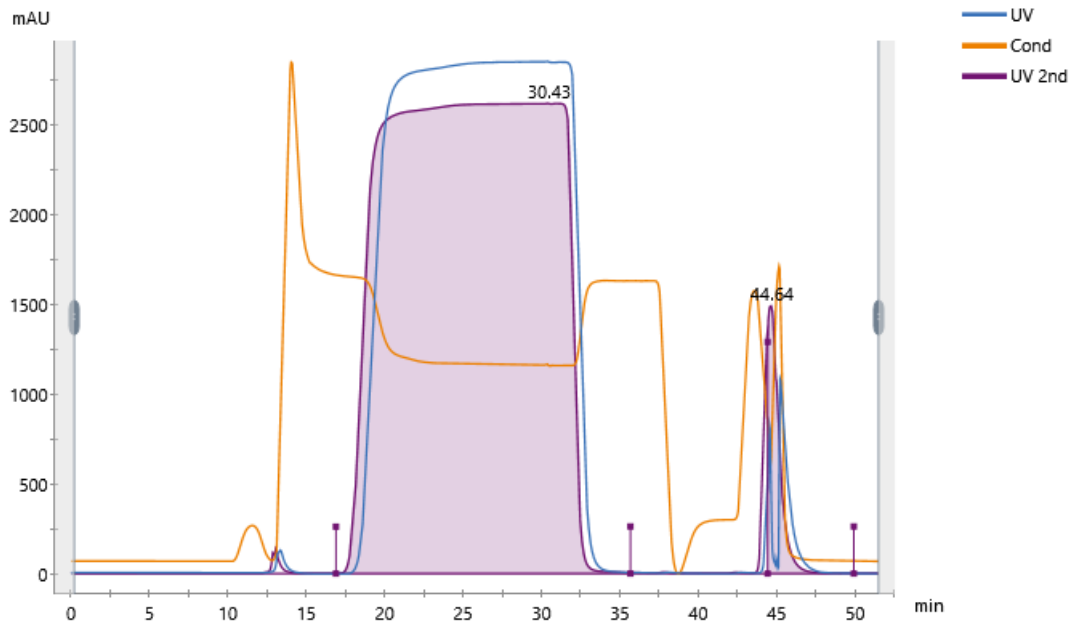

Figure 4.1.2. Successful pooling of Mabselect SuRe eluate. Wide purple peak is the sample load, second purple peak is the eluate, the darker purple peak fragment is the pool that the pooling function has cut off. This will be the SEC load. AC-UV (purple), SEC-UV (blue), conductivity (yellow).

Figure 4.1.2. shows the pooling function in action. This chromatogram contains the same phases as the previous figure did. There is a large purple peak in the middle which is the load peak and the second thinner purple peak is the elution peak. The elution peak has a darker purple cut off section in the middle which visualises the fraction of the peak that is to be loaded upon the SEC, in other words, the SEC load. This can be more clearly seen in figure 4.1.3 which is the same chromatogram but zoomed in. A large gap can be seen for the SEC-UV sensor in figure 4.1.3. More on this in section 5.1.

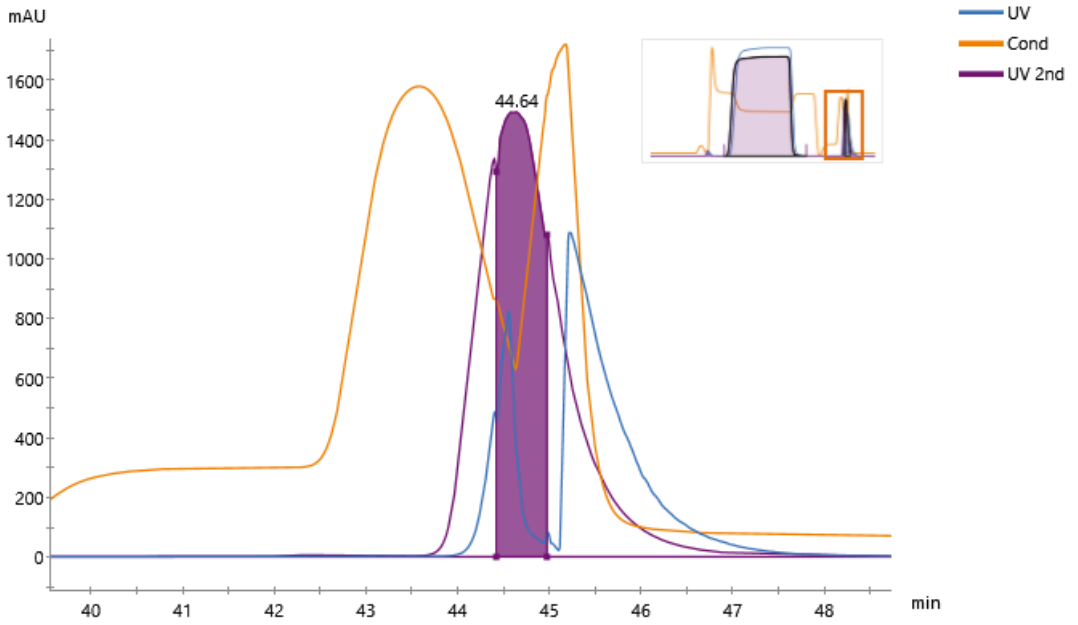

Figure 4.1.3.. Detailed successful pooling peak. Purple peak is the entire elution peak, the darker purple peak fragment is the pool that the pooling function has cut off. This will be the SEC load. AC-UV (purple), SEC-UV (blue), conductivity (yellow).

4.2 Single serial cycle

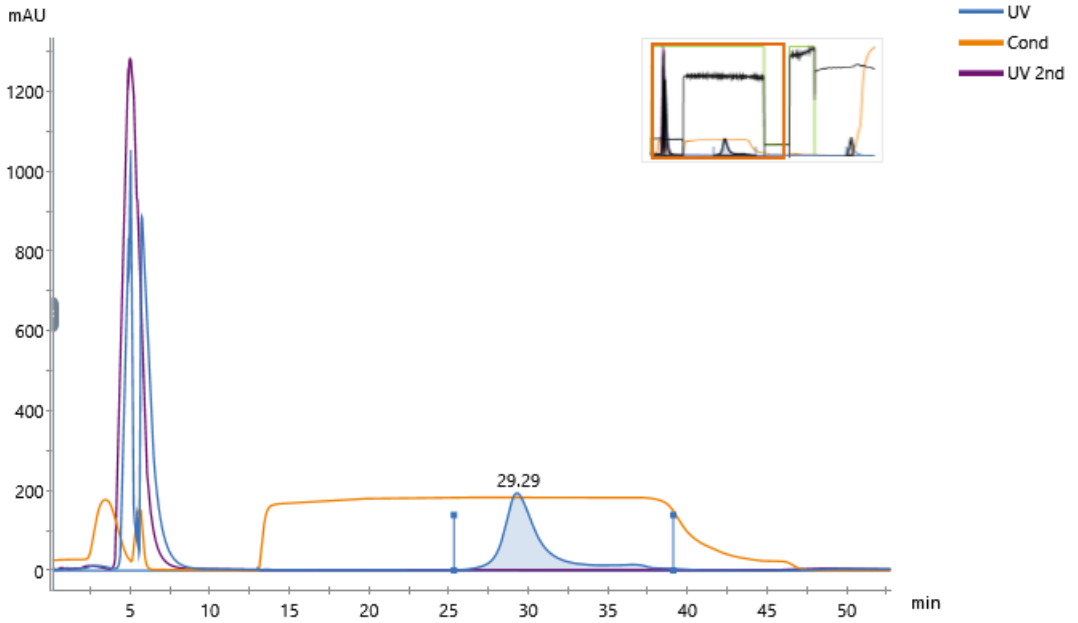

Figure 4.2.1. Successful pooling and loading of SEC. First joint blue and purple peak is the pooling, second blue peak is the wash from the SEC. The SEC was 2% loaded and washed with the pH 3.5 acetate buffer. AC-UV (purple), SEC-UV (blue), conductivity (yellow).

In figure 4.2.1, a successful SEC load can be seen. The pooling function worked and loaded the SEC. This is proved by there being a second blue peak following the pooling peak as this is the SEC wash peak. The SEC was loaded with 0.5 ml of pooled sample which is 2% of the total SEC volume. The SEC wash buffer is the same as the SuRe elution buffer, a pH 3.5 acetate

buffer. The pooling peak looks cluttered, but has actually the same appearance as in figure 4.1.3, the time scale is just larger.

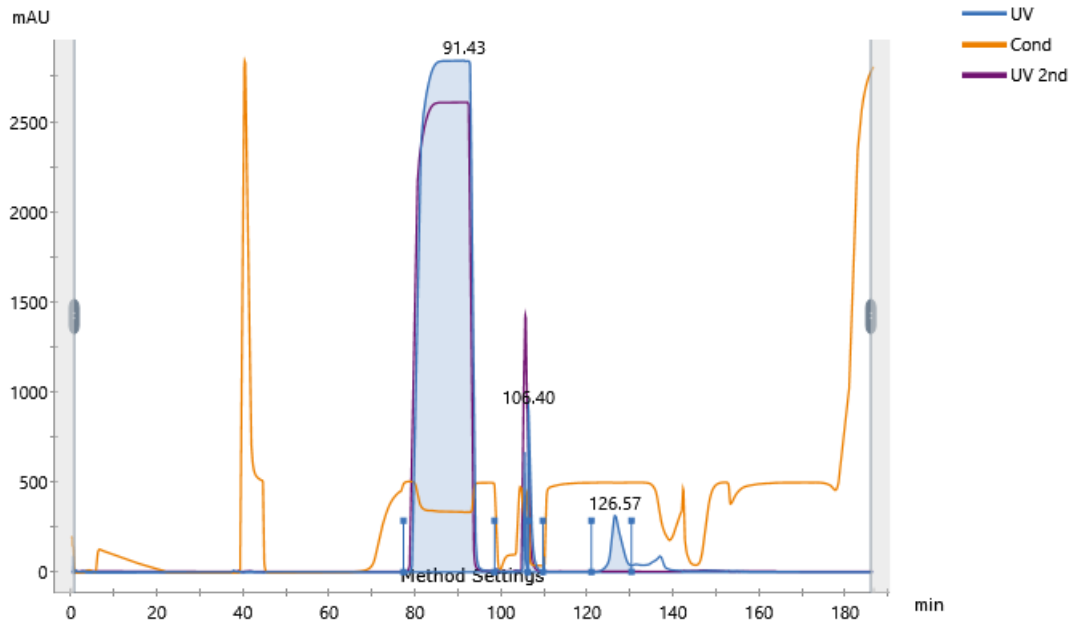

Figure 4.2.2. 2% SEC Load washed with pH 7.4 phosphate buffer. Large blue peak is sample load, second blue/purple peak is the SuRe eluate, third non-uniform peak is the SEC wash. AC-UV (purple), SEC-UV (blue), conductivity (yellow).

In figure 4.2.2 the SEC wash buffer has been changed to a pH 7.4 phosphate buffer which alters the appearance of the SEC wash peak. In addition, in this figure all three main steps can be seen starting from the left blue peak; the SuRe load, the SuRe elution/SEC load and finally the SEC wash.

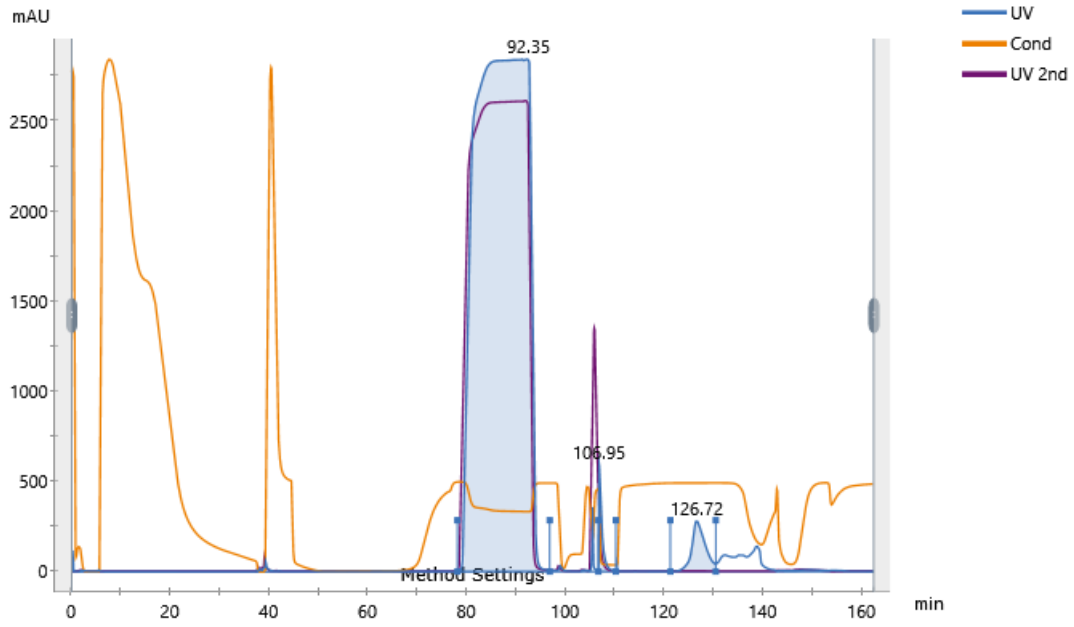

Figure 4.2.3. 5% SEC Load washed with pH 7.4 phosphate buffer. Large blue peak is sample load, second blue/purple peak is the SEC load, third non-uniform peak is the SEC wash. AC-UV (purple), SEC-UV (blue), conductivity (yellow).

In figure 4.2.3 the SEC was loaded with 1.2ml of pooled sample which is 5% of the total SEC volume. This alters the SEC wash peak slightly which is discussed in section 5.1. The SEC wash buffer is the same as the previous run in figure 4.2.2, pH 7.4 phosphate buffer. All three main steps can be seen in this figure.

4.3 Multiple serial cycles

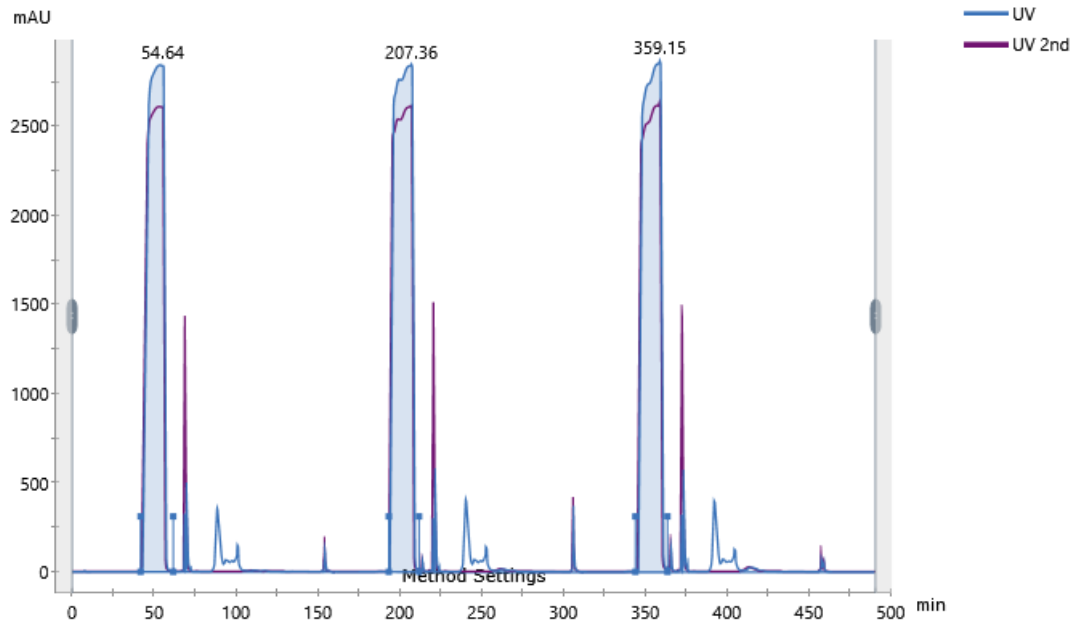

Figure 4.3.1. Multiple serial cycles. The SEC is 5% loaded and is washed with the pH 7.4 phosphate buffer. First large blue peak is the sample load, second thin blue/purple peak is the SEC load, the third non-uniform peak is the SEC wash. This pattern is repeated twice more. Total process time was 8.2h. AC-UV (purple), SEC-UV (blue).

Figure 4.3.1 shows the chromatogram for the multiple serial cycles test. In essence, it is the same chromatogram as in figure 4.2.3, but repeated three times. The three cycles are quite clear and all three major peaks can be seen for each cycle, the sample load, the SEC load and the SEC wash peaks.

Table 4.3.1. Cycle data overview of the multiple serial cycle run.

Cycle	***Sample load (mg)***	***SEC load (mg)***	***Affinity yield (%)***	***Product (mg)***	***SEC yield (%)***	***Cycle yield (%)***
1	*6.0*	*4.1*	*68*	*2.8*	*68*	*46*
2	*6.0*	*4.4*	*73*	*3.1*	*70*	*51*
3	*6.0*	*4.5*	*75*	*3.0*	*66*	*51*
Total	*18*	*13.02*	*72*	*8.8*	*68*	*49*

Accompanying the chromatogram of figure 4.3.1 is table 4.3.1 which gives data about the process. The data displayed is the sample load , the SEC load and amount of product produced (SEC wash). The different yields of each step are also displayed. The affinity yield is calculated by dividing the SEC load by the sample load. The SEC yield is the amount of product divided by the SEC load. The cycle yield is the amount of product divided by the sample load. All the

cycles yields are very similar to each other. The total in regards to weight is the sum of the cycles, while the total in regards to yields are the averages of all the cycles.

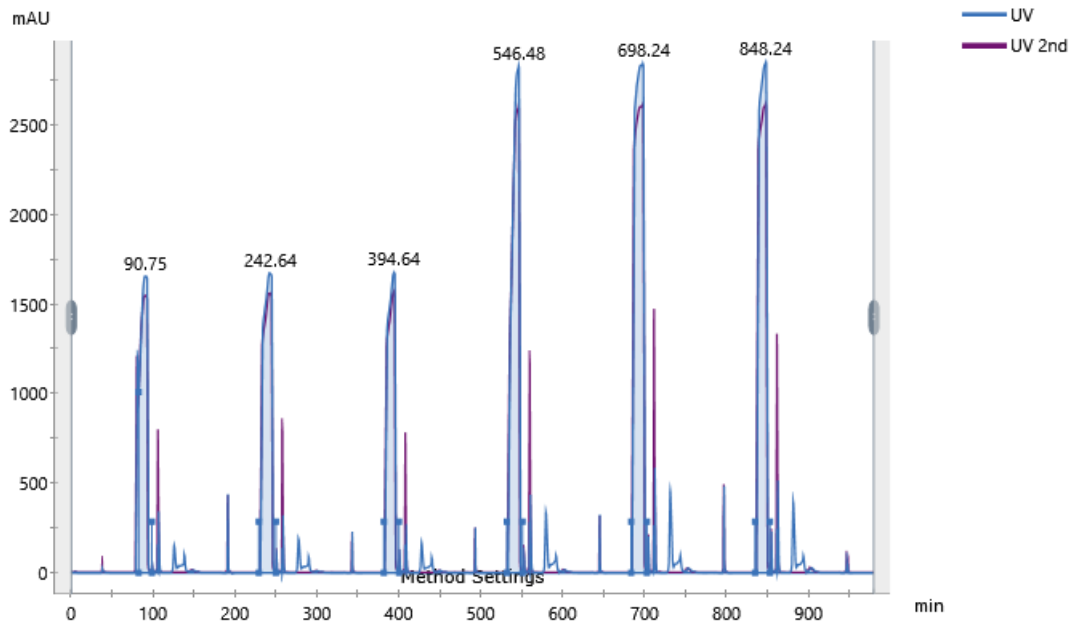

Figure 4.3.2. Multiple cycles per sample. 3 cycles were run per sample. The concentrations were 0.4mg/ml and 0.8mg/ml respectively. The first three cycles were loaded with the 0.4mg/ml sample and the three last cycles were loaded with the 0.8mg/ml. AC-UV (purple), SEC-UV (blue).

The chromatogram shown above as figure 4.3.2 is the first successful test of running different sample concentrations. The two sample concentrations were 0.4mg/ml and 0.8mg/ml of monoclonal antibodies. The first three cycles are loaded with the 0.4mg/ml and the last three are loaded with the 0.8mg/ml. The difference in concentration can be most easily seen with the sample load peaks for the 0.4mg/ml cycles, these peaks are about half of that of the following three cycles, the 0.8mg/ml cycles. The pattern of peaks is the same for each cycle, starting with the sample load, SEC load, and finally a SEC wash peak. With the current scale of the chromatogram, it can be difficult to see each peak in each of the 0.4mg/ml cycles, but the pattern is the same, the peaks just reach lower absorbance levels.

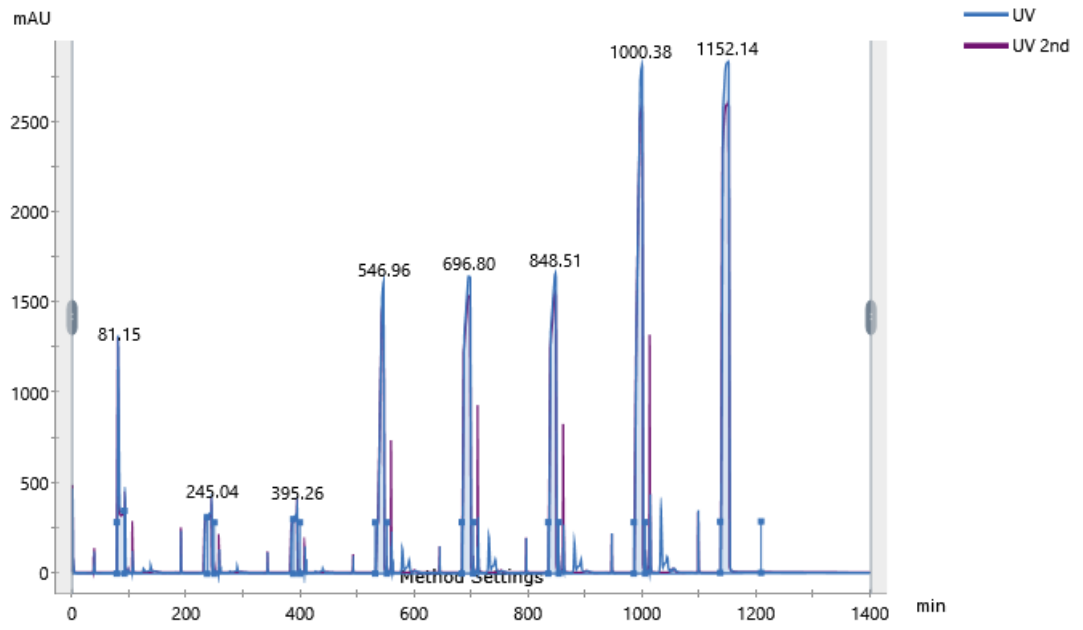

Figure 4.3.3. Multiple cycles per sample extended. 3 cycles were intended to run per sample, but only 7 complete cycles were completed due to connection error. The concentrations were 0.08mg/ml, 0.4mg/ml and 0.8mg/ml. The three first cycles are the 0.08mg/ml, the three subsequent ones are the 0.4mg/ml and the two last cycles are the 0.8mg/ml. AC-UV (purple), SEC-UV (blue).

The chromatogram of figure 4.3.3 is a continuation of the success of run in figure 4.3.2. This run was loaded with three different sample concentrations 0.08mg/ml, 0.4mg/ml and 0.8mg/ml of monoclonal antibodies. Three cycles were supposed to be run for each sample, making a supposed total number of cycles 9. The first three cycles are loaded with the 0.08mg/ml, the following three cycles are loaded with the 0.4mg/ml and the last two were loaded with 0.8mg/ml. Unfortunately, during the 8th cycle, a connection error occurred and stopped the process. The 0.08mg/ml peaks are the shortest and can be hard to see, the 0.4mg/ml peaks are a bit easier to see as they are taller than the 0.08mg/ml peaks by quite a lot and finally the tallest peaks are during the 0.8mg/ml cycles.

4.4 Single parallel cycle

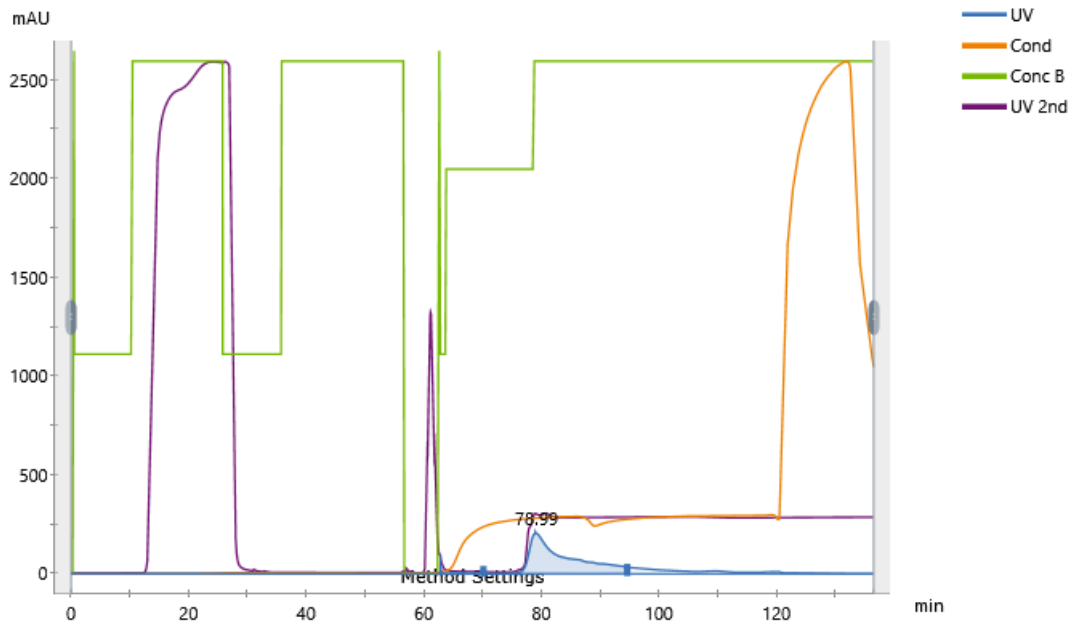

Figure 4.4.1. Successful single parallel cycle. The SEC was 5% loaded and washed with pH 7.4 phosphate buffer. AC-UV (purple), SEC-UV (blue), conductivity (yellow), percentage of B (green).

In figure 4.4.1 the first successful single parallel cycle can be seen. A new label has been added to this chromatogram, the percentage of B used which is the green line. This means how much of the system flow is made of pump B, which is the pump connected to the SEC. This helps in displaying when the different parallel phases are activated. An overview of the parallel phases can be seen in figure 3.2.3. Starting from the far left the green line is about 43%, here the SuRE (affinity) and SEC flush phases are both active with the affinity phases coming to a halt after a while and the percentage of B rushes up to 100% as the affinity column is loaded by the sample pump (the sample pump is not included in the total flow). Here is also the first purple peak.

Afterwards the percentage of B drops back to 43% as the affinity wash phases are activated whilst the SEC flush phase continues and later enter equilibration phase. As the affinity wash phases are quite brief, the affinity phases stops and waits for the SEC equilibration phase to be completed.

Once the SEC equilibration phase is done, the two branches merges into the elution/pooling phase. Here the percentage of B drops to 0% as this phase is completely controlled by pump A. Here the second purple peak is the SEC load.

Thereafter, the affinity column enters the CIP and regeneration phases and just as before, these phases together are a lot shorter than the SEC phases and eventually the percentage of B reaches 100% as the affinity phases stops and waits for the SEC to complete all its phases. Unfortunately, a last affinity flush phase was forgotten to be added therefore the purple CIP peak drags on for quite a while as there is no flow through the affinity column. A final affinity flush phase was added to all subsequent parallel runs.

Right after the elution phase is done the SEC wash phase starts, where also the blue SEC wash peak can be seen, followed by a CIP phase and lastly a flush phase.

4.5 Multiple parallel cycles

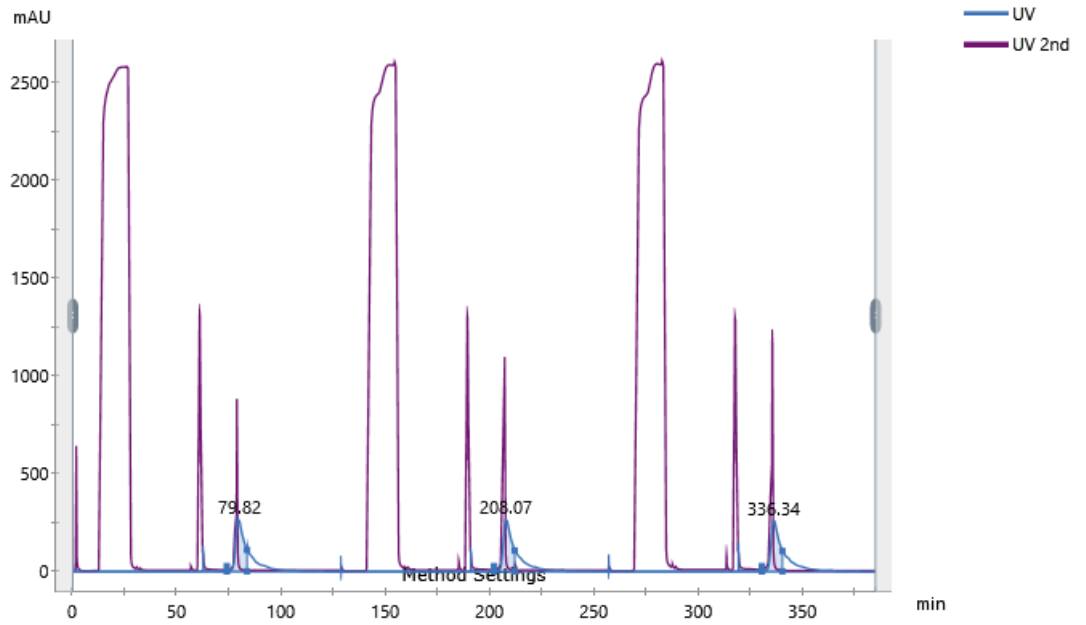

Figure 4.5.1. Successful multiple parallel cycles. 3 cycles were performed. The SEC was 5% loaded and washed with pH 7.4 phosphate buffer. Total process time was 6.4h. AC-UV (purple), SEC-UV (blue).

In figure 4.5.1, three successful cycles of the parallel system can be seen. The pattern of one cycle is the same as in figure 4.4.1 except the CIP peak has been successfully removed by adding an affinity flush phase at the end of each cycle. Total process time was 6.4h.

*Table 4.5.1. Comparative data overview of the two multiple cycles runs. * - due to the nature of the SEC wash peaks in figure A3, the cut offs are not reliable but to illustrate the productivity potential, it is assumed that the total product weight is the same as for the serial process. A more comprehensive overview can be seen in table A2.*

Process	***Process Time (h)***	***Total Product (mg)***	***Process yield (%)***	***Productivity (mg/h)***
Serial	*8.2*	*8.8*	*48*	*1.07*
Parallel	*6.4*	*8.8**	*48**	*1.38**

A comparison between the data obtained from the multiple serial run in figure 4.3.1 and the multiple parallel run in figure 4.5.1 can be seen in table 4.5.1. The difference in process time is 1.8h. The main result from this table is the productivity in mg/h. The parallel process is roughly 30% more productive. An asterisk had to be added to the parallel data as it is assumed that this process yields the same amount of product as the serial run. This assumption is needed since the cut offs in figure 4.5.1 are quite unreliable because there is no clear separation between the monomers and the subsequent fragments.

5 Discussion

5.1 Process implementation

The initial Mabselect SuRe elution tests of figure 4.1.1 and figure 4.1.2 looked like they should have. The first peak in figures 4.1.1 and 4.1.2 is the load peak which of course will be broader than the elution peak. This is due to the fact that it is a supernatant which is being loaded upon the system and in it there is a lot more organic matter than just antibodies such as cell debris, that is being detected by the UV-sensors. The elution peak is a lot thinner but its maximum absorbance is quite high, about 1400mAU, as this a very concentrated eluate. The total eluate of the SuRe is about 5ml.

The subsequent pooling results of figures 4.1.2 and 4.1.3 seems to have worked sufficiently. The SEC load is very close to 0.5 ml and a clear cut off in the purple section can be seen in the more detailed figure of 4.1.3 to illustrate the SEC load pool. An interesting phenomenon can be seen in figure 4.1.3 with the purple and blue lines respectively. Since the purple lines is the absorbance before the SEC (AC-UV) and the blue lines is the absorbance after the SEC (SEC-UV), the SEC column is loaded (or in the case of figure 4.1.3 as no SEC was connected, waste was used instead) there will naturally be no absorbance read by the SEC-UV sensor. This is because the particular amount of pooled antibodies will not pass through the SEC-UV sensor, only the AC-UV sensor, leaving a noticeable gap for the blue peak.

Once the SEC was connected to the system, a complete pooling and SEC wash could finally be run and seen, which figure 4.2.1 illustrates. The first peak is the pooled SEC load and the smaller blue peak is the SEC wash which is the final purified monoclonal antibody. The SEC wash peak is relatively uniform as well as broad with a long tail that could indicate that there are smaller antibody fragments in the supernatant [11]. No larger aggregates could be seen which would have washed out earlier, but as the peak was not further analysed with for example SDS-PAGE, it is impossible to say that there is no aggregates present [12].

A more comprehensive view of a complete single serial cycle is seen in figure 4.2.2 which includes the load peak as well as the SEC load and SEC wash peaks. Here the SEC wash peak is a lot more non-uniform to that of the previous run. This was because the pH of the SEC wash buffer was increased from 3.5 to 7.4 which made the peak's tail longer [13]. The first peak is the monomer monoclonal antibody (which is highlighted), and the much longer tail is probably further indication of antibody fragments being present [11]. The SEC in the runs of figures 4.2.1 and 4.2.2 was loaded with 2% (0.5 ml of affinity eluate). According to Cytiva this is the maximum load, but some industry practices use 5% (1.2ml of affinity eluate). Therefore, the subsequent run of figure 4.2.3 was loaded with 5%. The most noticeable difference is the fragmented part of the SEC wash peak that is wider and broader and arguably the resolution is worse.

The multiple serial cycles run was seen as a success. Three almost identical cycles can be seen in figure 4.3.1 and shows promise that the process can be run for an extended period of time. The accompanying table 4.3.1 displays that the cycle yields are very similar to each other, meaning the process is quite robust and reliable. This also indicates that the wash and CIP phases of each cycle cleans the system enough so that the next cycle run doesn't lose any production capacity. Note that only 3 cycles were made and a lot more cycles would have probably been needed to run if any capacity is to be lost. Unfortunately, the cycle yields are quite low, never reaching more than 51%. However, if this process were to be scaled up, the need for higher yields is outmatched by the system being more robust thus being able to predict how

much can be produced as the yields are more or less constant. Higher yields could be improved in future prospects for example by switching to the Mabselect PrismA or using periodic counter-current affinity chromatography (PCC).

The multiple serial cycles tests with varied concentrations did however face some challenges. As these tests were to be run for the longest duration, 6 cycles and 9 cycles, meaning about 16h and 24h respectively, they were most likely to run into connection errors. The tests before had occasionally run into some connection error between Orbit and Unicorn, but was for the most part easily fixed as the runs were only a few hours long. The connection error is a known problem with Orbit when using the Unicorn OPC interface, fortunately changing the connection type to the more robust Unicorn REST API interface improves stability to the connection. This was done after some initial tests failed while running the varied concentration script. The two-concentrations test worked excellent and provided a well looking chromatogram of figure 4.3.2 with the three first cycles being a lower concentration, 0.4mg/ml and the three last being the standard concentration of 0.8mg/ml. This means that the script worked well both in test and real mode. The three-concentrations test did work for some time, but 8 cycles in, a connection error occurred and the run couldn't be saved. However, if looked through a more "proof of concept" view, the system shows promise of being run at extended periods of time with different sample batches. The chromatogram of figure 4.3.3 clearly shows the effects on the increased concentrations on the process such as higher load, SEC load and SEC wash peaks and could be reminiscent to an actual industrial chromatogram.

The concentration variable of the last tests could be replaced by a time variable in the case of an unknown antibody concentration sample. This would replace the improved pooling function mentioned in section 3.1.5 by just inserting a time when the antibody is believed to be eluted from the SuRe. The point of having a concentration variable is to be more certain of capturing the highest concentrated fractions from the SuRe elution, thus increasing the affinity yield. This could be useful for industries where regularly cultivated batches are used and most concentrations are known.

5.2 Comparing serial & parallel processes

Overall the parallel implementation went well. There were some difficulties getting the parallel methods working such as the two system pumps being used simultaneously but being set individually. The chromatograms in figures 4.4.1 and 4.5.1 are slightly more difficult to read and aren't as clear as its serial counterparts (the chromatogram in figure 4.4.1 is explained in detail in section 4.4). In addition, it is unfortunately quite difficult to make an adequate cut off of the SEC wash peak in figure 4.4.1 as the peak has a very tail-like characteristic. This is also the case for all SEC wash peaks in the multiple cycle test in figure 4.5.1 One underlying cause could be either the overloading of the SEC or having a high pH for the SEC wash buffer. Another possibility is the fact that two different supernatant samples were used for the serial and the parallel runs respectively and there can be slight deviations in the amount of fragments present in each sample. Regardless, an additional test of using the pH 3.5 SEC wash buffer could have shown greater separation and aided in the cut off, but then the opportunity to study the monoclonal antibody in a natural human pH is lost and it may also be denatured [13]. Therefore, one way to proceed is to fractionalize the peak and analyse the different fractions to find the one with the most intact monoclonal antibodies.

In table 4.5.1 a comparative overview of figures 4.5.1 and 4.3.1 shows the differences in process time and productivity between the serial and parallel runs. As mentioned before, the cut off is

uncertain but to illustrate the potential difference in productivity, it is assumed that the same amount of monoclonal antibodies is acquired from the parallel run as in the serial run.
Using this assumption, the parallel process is actually about 30% more productive. If time is a limiting factor for a certain industrial monoclonal antibody purification process, this parallel process concept could be advantageous for mitigating time issues. It certainly needs optimizing and fine-tuning, but regardless, a 30% productivity increase could be beneficial in times where monoclonal antibody supply is in high demand.

5.3 Future process improvement

The biggest drawback of this serial implementation is the fact that only small sample volumes were tested, 7.5ml. At a preparative scale, the system has to be able to handle large volumes. Therefore, it would be very beneficial to test the system with a wide range of sample volumes such as 10, 30, 50 and even 100ml. This would mean that the dynamic binding capacity of the Mabselect SuRe would be much more utilized. As mentioned before, one could even replace the Mabselect SuRe to Mabselect PrismA as this column has a better dynamic binding capacity as seen in figure 3.1 and would improve capture yield.

In addition to scaling up the sample volumes, additional samples could also be implemented. In its current state, the serial system can take 7 samples using the sample valve. However, since the ÄKTA pure is highly configurable and it is possible to connect multiple inlet valves with each other, one could add the inlet valve X1 to the sample valve, increasing the sample capacity to 14. Now, the interesting part is that Orbit is not limited to the same restrictions as Unicorn, therefore it is possible to add even more inlet valves upon the already two active ones. This means, in theory, the sample capacity is limited to how many inlet valves one could get hold off. However, 14 samples on one ÄKTA pure is still very plentiful.

The parallel implementation can also be further improved. Whilst a 30% productivity increase is no small feat, the SEC is still a noticeable bottleneck of the system. This means that the SuRe is not being fully utilized and placed in wait mode while some of the SEC phases are being run. One way to improve this system is by adding a second SEC to the system in its own flow path. The system would then have three parallel flow paths and extensively use all three pumps. An overview of this triple parallel system can be seen in figure 5.3. One pump for the samples and affinity buffers and two pumps for SEC buffers. Using a more intricate system of versatile valves it is possible to connect both SECs to the SuRe. A versatile valve also has to be used as a loop valve as it is only possible to connect one loop valve per system. Ideally, 3 column valves should be used as these have built in pressure sensors and could be used with some tinkering in Orbit, but for simplicity's sake, versatile valves and loop valves will do fine [7]. High pressures could be an issue in the system. The UV sensor named UV1 will most likely experience high pressures as there are a lot of modules before it, so maybe flow rates have to be lowered somewhat as well.

A branched phase list of this system can be found in figure A.1. As this branched phase list is also a three-cycle process, it can be directly compared to the two other runs, the multiple serial run and the multiple parallel run. The total process time of the triple parallel process is 230 min, but keep in mind that this is using the same parameters as in dual parallel process. Regardless it is a substantial decrease in process time. If one could yield an equal amount product as in the serial run, 8.8mg, and let's assume this to be true for a moment, the productivity would thus become an impressive 2.30 mg/h. This is a 66% increase of the assumed dual parallel productivity in table 4.5.1 and a 115% increase compared to the serial productivity. Note that this first

has to be implemented and optimized, but this really shows that there are great opportunities in turning serial purification processes into parallel ones.

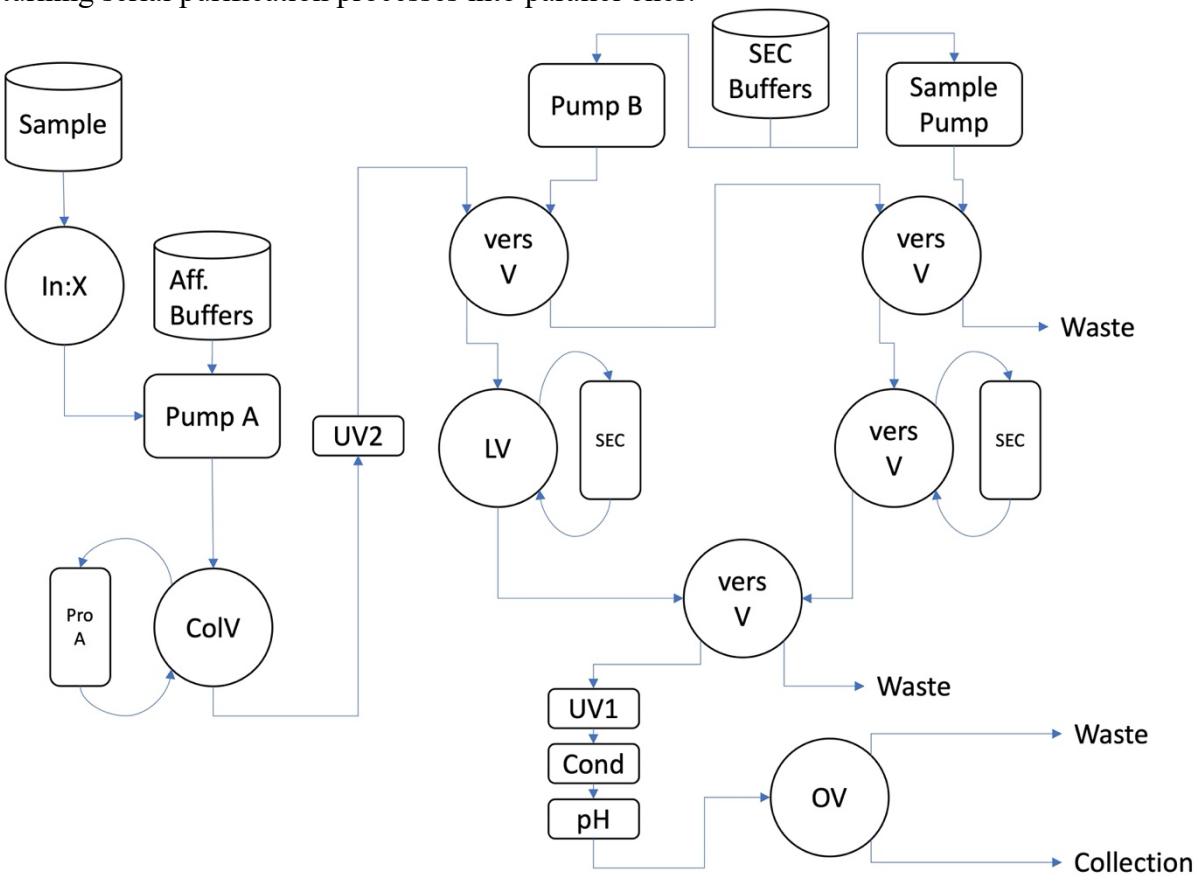

Figure 5.3. Triple parallel flow path concept.

5.4 ÄKTA pure, a replacement of ÄKTAxpress?

The ÄKTAxpress system is a reliable chromatography system, but compared to the ÄKTA pure, it is becoming clearer that the ÄKTAxpress is a bit outdated and the potentials of ÄKTA pure are vastly numerous. The ÄKTAxpress can only take 4 samples, while the ÄKTA pure can in practice take up to 7 or 14 samples, but in theory much more. This would mean that the ÄKTA pure could run much more autonomously and for longer periods of time, being run for well above a weekend, maybe even up to a full week. In theory it could be run for so much longer, as long as there are samples to be loaded and enough buffers. The ÄKTA pure's production limit is its fraction collector, even though the ÄKTA pure's fraction collector can take up to six 96-well plates instead of ÄKTAxpress' single one. Six 96-well plates would in practice mean that the production limit for ÄKTA pure is probably around 12 days, using the information that one plate is roughly filled up in 2 days of continuous use. This is alone is a significant improvement compared to the ÄKTAxpress. It is important to note though, that all the implementation that has been done in this project are on a small scale. A scale up is needed before the actual time limit of the process can be validated.

A parallel implementation is not essential for monoclonal antibody production, but could be as mentioned a powerful method in increasing production in times of need. It does come with a downside and it is its increased complexity. Unicorn comes with great visual aid for the system being run, but if more and more control is set to Orbit, Unicorn's visual aids will become redundant and an operator would have to have quite a lot of knowledge and experience in the

ongoing parallel process. This is the reaons why a serial implementation is probably the first step in trying to replace the ÄKTAxpress. However, one could try to integrate some visual aid into Orbit and the parallel system as Python has plenty of visualisation modules.

6 Conclusion

In conclusion, as the ÄKTAxpress is being discontinued, this ÄKTA pure system with some optimization and scale up, would be a suitable replacement for the ÄKTAxpress, or at least work as an interim system. It is certainly able to perform the same purification process with an affinity column followed by a size exclusion chromatography column. In addition, the ÄKTA pure is a highly configurable and customisable system and it works excellent with external control systems such as Orbit to perform more advanced methods such as determining the best pooling cut off using sample concentrations, automated sample changes depending on sample volumes and running the process with parallel flow paths. These methods are desired by the industry and should be taken in consideration when an eventual chromatography system will replace the ÄKTAxpress.

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8 Appendix

Table A1. Amount of product acquired from the SEC wash peaks in the multiple serial run (figure 4.3.1).

Peak	***Peak area (ml*mAU)***	***Extinction coefficient (mg*ml^{-1}*cm^{-1})***	***Amount (mg)***	***Concentration (mg/ml)***
First cycle SEC wash peak	769	1.4	2.747	0.43
Second cycle SEC wash peak	864.5	1.4	3.087	0.483
Third cycle SEC wash peak	881.8	1.4	3.149	0.512

*Table A.2 Comprehensive data overview of the two multiple cycles runs. * - due to the nature of the SEC wash peaks in figure 4.5.1, the cut offs are not reliable and thus it is assumed that the total product weight is the same as for the serial process.*

Process	***Time (h)***	***Total load volume (ml)***	***Total load (mg)***	***Total product (mg)***	***Process yield (%)***	***Productivity (mg/h)***
Serial	*8.2*	*22.5*	*18*	*8.8*	*48*	*1.03*
Parallel	*6.4*	*22.5*	*18*	*8.8**	*48**	*1.38**

Triple parallel			
Minute	Affinity	SEC1	SEC2
1	Flush	Flush	Flush
2			
3			
4			
5			
6	Eq		
7			
8			
9			
10			
11	Load		
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25		Eq	Eq
26	wash1		
27			
28			
29			
30			
31	wash2		
32			
33			
34			
35			
36	Wait		
37			
38			
39			
40			
41			
42			
43			
44			
45			
46			
47			
48			
49			
50			
51			
52			
53			
54			
55			
56			
57	Elu		Wait
58			
59			
60			
61			
62			
63			
64			
65	CIP	Wash	
66			
67			
68			
69			
70			
71			
72			
73			
74			
75	Reg		
76			
77	Flush		
78			
79			
80			
81			
82	Eq		
83			
84			
85			
86	Load		
87			
88			
89		CIP	
90			
91			
92			
93			
94			
95			
96			
97			
98			
99		Flush	
100			
101	wash1		
102			
103			
104			
105			
106	wash2		
107			
108			
109			
110			
111	Elu		Elu
112			
113			
114			
115			
116			
117			
118			
119	CIP		Wash
120			
121			
122			
123		Equi	
124			
125			
126			
127			
128			
129	Reg		
130			
131	Flush		
132			
133			
134			
135			
136	Eq		
137			
138			
139			
140	Load		
141			
142			
143			CIP
144			
145			
146			
147			
148			
149			
150			
151			
152			
153			Flush
154			
155	wash1	Wait	
156			
157			
158			
159			
160	wash2		
161			
162			
163			
164			
165	Elu		
166			
167			
168			
169			
170			
171			
172			
173	CIP	Wash	
174			
175			
176			
177			
178			
179			
180			
181			
182			
183	Reg		
184			
185	Flush		
186			
187			
188			
189			
190			
191			
192			
193			
194			
195			
196			
197		CIP	
198			
199			
200			
201			
202			
203			
204			
205			
206			
207		Flush	
208			
209			
210			
211			
212			
213			
214			
215			
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222			
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230			

Figure A.1 Branched phase list for a triple parallel flow path system minute by minute. The first column is the time, the second column is the affinity phases, the third column is the first SEC phases and the fourth column is the second SEC phases. Left: The phases for the first 114min. Right: The phases for the remaining 116min. Total process time is 230min.

Table A.3. Recipe for buffers used.

Buffer	**H_2O (ml)**	**NaH_2PO_4 (g)**	**Na_2HPO_4 (g)**	**NaCl (g)**	**NaOH (g)**	**$C_2H_3NaO_2$ (g)**	**CH_3COOH (ml)**
20mM Na-Phos-phate 150mM NaCl pH 7.4	800+200	1.07	1.56	8.766	-	-	-
50mM Na-Ace-tate pH 6	800+200	-	-	-	-	5.17	0.6
50mM Na-Ace-tate pH 3.5	800+200	-	-	-	-	1.49	2.2
1.0M NaOH	400+100	-	-	-	20	-	-
0.1M NaOH	400+100	-	-	-	2	-	-

9 Populärvetenskaplig artikel

Småskalig plattform för upprening av monoklonala antikroppar

Utvecklingen av en uppreningsplatform för antikroppar visar en 30% ökning i produktivitet med hjälp av parallella flödesbanor. Det är nu också möjligt att upprena ännu fler prov per körning än tidigare.

Utvecklingen av nya antikroppar blir allt mer viktigare i dagens samhälle för att kunna förbättra vår analytiska och diagnostiska förmåga. Innan dessa nya antikroppar kommer vidare till den kliniska fasen där de kan godkännas för marknaden, måste de utvärderas och bli testade. För att detta ska bli möjligt, måste man producera en liten mängd antikroppar. Detta görs med hjälp av en uppreningsprocess som ska helst vara snabb, billig och pålitlig eftersom risken finns att just en antikroppsvariant inte blir godkänd och då vill man inte slösa allt för mycket resurser.
Reningsprocessen här utgörs av två steg, först affinitetskromatografi följt av gelfiltrering. Affinitetskromatografsteget fångar upp antikropparna från en lösning och gelfiltreringen filtrerar de uppfångade antikropparna med avseende på storlek. Processen körs på särskilda kromatografisystem, och nästa år, 2023, utgår faktiskt ett av de vanligaste kromatografisystem som kallas ÄKTAXPRESS. Då behövs iallafall ett kortsiktigt ersättningssystem tills ett nytt system har utvecklats, och här finns det en unik chans i att belysa industrins framtida behov.

I detta examensarbete överfördes och implementerades denna uppreningsprocess på ett annat kromatografisystem, ÄKTA PURE, som är väldigt anpassningsbart och då kunde att nya metoder utvecklas med hjälp av det Python-baserade styrsystemet Orbit. Resultatet var framgångsrikt och det visade sig att det nya systemet kunde köra samma uppreningsprocess som ÄKTAXPRESS. Här utvecklades bland annat nya metoder för att kunna upprena betydligt fler prover per körning samt smartare interaktioner mellan de två processtegen.

Utöver detta, utvecklades ytterligare ett koncept på ÄKTA PURE som kunde köra vissa delar av processen i parallella flödesbanor så att man kunde minska körningstiden och på så sätt öka produktiviteten. Detta koncept belyste potentialen med att köra vissa steg parallellt då ett praktiskt exempel visade en 30% ökning av produktiviteten. En sådan ökning av produktivitet är förmånligt vid stora produktionsbehov av antikroppar.