Modelling and Simulation of Antibody Purification

By

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Abstract

Due to their high specificity against target antigens, monoclonal antibodies (mAbs) have proven to be a very powerful tool for diagnostic and therapeutic purpose against several diseases. Currently there are over 26 mAb approved for therapeutic use by the FDA. The manufacture of mAbs is a complex process where purification plays a key role. This project proposes and simulates a purification process based on an array of chromatographic columns. The different employed columns were modelled by kinetic dispersive models using parameters from available bibliography as well as some assumptions. The proposed method consists of five columns connected in series and offered arguably good results with a purity and yield of the target mAb of~90% and ~85% respectively.

Summary

Monoclonal antibodies are a group of molecules with growing interest in the pharmaceutical industry. The different variations of this biomolecules are capable of identifying and binding to a wide variety of targets such as viruses, cancer cells or other agents causing diseases. This makes monoclonal antibodies a very powerful tool for diagnosis and therapy of diseases.

One of the critical steps in the manufacture of monoclonal antibodies is the purification process. The purpose of this work is to define a purification process and simulate it to predict the expected results.

In order to purify the target antibody it must be separated from the impurities present in the initial solution, which is achieved by using chromatographic columns which retain the different components more or less time depending on physical and chemical interactions, this means that some components will leave the column earlier while others will take longer allowing us the separate the target antibody from the impurities.

These different columns separate the components on the basis of their different charge, affinity to bind to a biomolecule and the different size of the components.

The simulated purification process consists of five different columns connected in series where the outflow of one column continuously feeds the next one.

This simulation showed promising results as over 90% purity was reached with a high yield of product and there is margin to refine the process.

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1. Introduction

1.1. Aim

The aim of this work is to define, model and simulate a method of purification of monoclonal immunoglobulin G (IgG). The entire process is to be based on chromatographic methods and the solution is expected to produce a good yield of IgG with high purity.

1.2. Immunoglobulin G

Antibodies or immunoglobulin are Y-shaped proteins which form part of the immune system. Their role is to identify and neutralize pathogens. They are rather big proteins with a molecular weight that can be estimated to 150kDa.

All the immunoglobulins have a common structure with two identical heavy chains and two identical light chains. A disulphide bond, binds each light chain with one of the heavy chains, the heavy chains are also joint together by disulphide bonds [1].

The molecule can be divided into two fragments, the Fab-fragments which contains variable region of the light chains and the heavy chains, and the Fc-fragment.

The tip at the end of the heavy and light chains in the Fab-fragment combine to form two identical antigen binding sites, this is the part of the antibody that binds to the target antigen. This variable region allows for many possibilities thus the immune system is capable identify a wide range of antigens due to the great diversity of antibodies. The part of the antigen where the antibody binds is named epitope [1].

Depending on the heavy chains of the antibody they can be classified into isotypes. The isotype which is object of this study is IgG. This immunoglobulin is the most abundant antibody found in humans and they are generated by plasma cells.

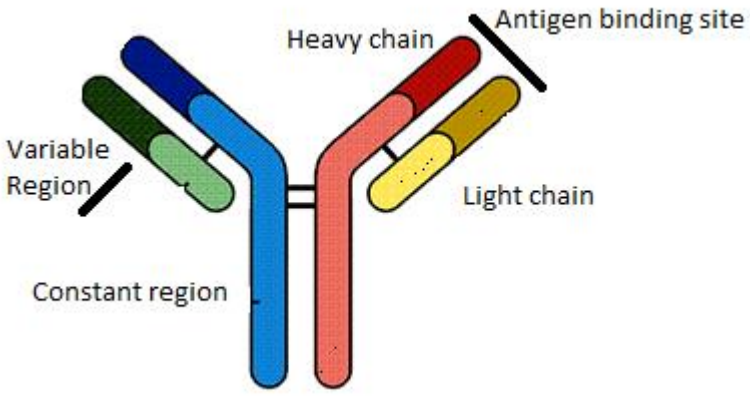

Figure 1 Immunoglobulin G structure

The purpose of this project is purifying monoclonal IgG. Monoclonal antibodies (mAbs) are those who are made by identical immune cells. The distinct feature of mAbs is that they have affinity to a single antigen this means they can target disease causing organism with high selectivity. This means different mAbs can be used to treat or diagnose a wide range of diseases such as cancer or auto immune diseases.

1.2.1. Aggregation

Protein aggregation is a phenomenon that occurs during the different steps of protein production and affects the quality, safety and efficacy of the product. The mechanism of aggregation is poorly understood [2]. These aggregates appear initially as dimers and then grow into larger structures.

The difference between the biological activity between the aggregates and the monomeric protein can significantly affect the potency of the protein based drug [3]. Also proteins aggregates can result in immune responses to the therapeutic product. [4].

There are no defined limits for the maximum allowable aggregate levels in protein-based drugs since the effect of these aggregates on final product depends on the product itself. The only limitation described in the US pharmacopeia and European pharmacopeia refers to sub-visible particles "Solutions for injection must be clear and practically free from particles". [2] Therefore those limits must be specified for each product drug.

As they are proteins mAbs also suffer from aggregation. Aggregates can form during the various steps of monoclonal antibody manufacturing processes.

1.2.1.1. Aggregation during cell culture

Cell culture is the step where cells are grown to produce target molecule. Aggregation in this step is a common phenomenon; the aggregate levels during this step for monoclonal antibodies have been reported up to 30% in some cases [6].

1.2.1.2. Aggregates during purification

In order to purify monoclonal antibodies they go through a variety of steps based on different methods: this incur in variations of pH, ionic strength, concentration but the proteins also suffer mechanical stresses and are in contact with different materials. All of this has different effects on protein aggregation.

- **pH conditions:** A widely used step in antibody purification is protein A chromatography. This method requires a low pH. At low pH proteins might undergo structural changes that could contribute to product aggregation [7]. Another during the antibody purification that requires low pH is the virus inactivation step.
- **Salt buffers:** Salt buffers have complex effects on protein stability. Depending on the concentration, salt type and ionic strength this might have a stabilizing or destabilizing effect on the protein.
- **Agitation** also increases the effect of aggregation.
- **Pumping:** During the purification of the antibodies the use of pumps is quite extensive especially in steps that require flow control such as chromatography [2]. This leads to mechanical stresses that might enhance the effect of protein aggregation.
- **Ultrafiltration, Final filling, Freeze-Thaw and Storage:** During the last steps of the manufacture aggregation might also occur and since the aggregates are to be removed in the previous purification steps it's important to reduce the formation of aggregates in these last steps.

1.2.1.3. Aggregate removal by means of chromatography

Chromatography is the mean by which aggregates are to be removed. The first chromatographic column in the antibody purification process is normally an affinity chromatography column. This step is not capable of removing aggregates since they might bind to the column similarly to antibody.

The methods that have been demonstrated useful for the removal of aggregates are anionic and cationic ion exchange [2].

As the aggregates form bigger molecules than the monomer, size exclusion chromatography might also be a valid method for the removal of aggregates, but it is considered inefficient due the poor resolution of aggregates from the monomer. [8].

It is also possible to separate aggregates based on hydrophobicity using hydrophobic interaction chromatography methods, as the hydrophobicity of the antibody molecules increases with aggregation [2].

2. Separation methods

2.1. Non-chromatographic methods

Even though, the process described in this work is based entirely in chromatographic methods it might also be worth mentioning the trends in the different non-chromatographic methods that are employed in antibody purification processes.

2.1.1. Precipitation

Monoclonal antibodies can be purified by precipitation; this process is based on the addition of polyethylene glycol (PEG) and ammonium sulphate to separate the mAb from the liquid phase. This is an interesting method since it concentrating an antibody with such a method offers the best volume reduction any purification method can offer.

This process is used in lab-scale purification of IgG from bovine, human serum and mammalian cell culture supernatants [9].

2.1.2. Liquid-Liquid extraction

Liquid-Liquid extraction is a technique based on transferring objective antibody from one solvent into another. This method can be used as IgG has a tendency to transfer from an aqueous solutions into a hydrophobic PEG-rich phase, this can achieve a purity of IgG up to 70-95% and a yield of IgG higher than 95% [9].

2.1.3. High performance tangential flow filtration

A method proposed by van Reis [10] consist on a charged ultrafiltration (100-300kDa) that can be used to purify IgG in acidic conditions, the proposed membrane would repel the positively charged IgG even if it were able to pass the membrane.

2.2. Chromatographic methods

2.2.1. Fluidized bed and fixed bed chromatography

In a fluidized bed chromatography the particles are dispersed in a liquid medium as opposed to fixed bed chromatography. Operation of fluidized bed is more complex than that of fixed bed but it has the benefit that it allows the purification from unclarified feed stocks such as the cell culture media or fermentation broths [11].

On the other hand fixed bed chromatography remains the most widely used method [9]. Applications are normally run with a single bed whose dimensions can be increased if more capacity is required. These report simulated fixed be chromatography columns as these are less complex systems and it was assumed that the feed stock was previously clarified.

2.2.2. Size exclusion chromatography

Size exclusion is purification method based on the different size on the molecules to be separated. A size exclusion column is filled with porous particles of a determined pore size, which means that if the pore size is small enough it should allow separating molecules between those big enough to fit into the pores and those that cannot enter them. The smaller particles will diffuse through more space into the column and therefore will have a higher retention time.

Since monoclonal antibodies such as IgG have large molecular weight (about 150kDa) size exclusion is an effective method to separate these particles from smaller impurities. The problem with the size exclusion method is that since it is not an adsorption based method the productivity is low, and feed volumes are also required to be low >4%(column volume)[12] in order to produce effective separation.

Due to the different size nature of antibodies and its aggregates it could be expected that size exclusion (SEC) would be a good method to separate them but this process offers low productivity [9] and resolution [8].

In this project however, size exclusion chromatography columns are used in the purification process in order to increase the pH after the protein A column and to reduce the salt content after the Ion exchange column.

2.2.3. Anionic ion exchange chromatography

Depending on the product to be purified anionic ion exchange (AIEX) column can be run two different modes. For example Murine IgGs are run in bind-elute mode since good binding conditions can be achieved at pH 8-8.5 , for Human and chimeric IgGs flow-through mode is used instead since they do not bind to the column[9].

In this project anionic ion exchange was used as the last step in flow-through mode in order to eliminate the remaining aggregate concentration, as the aggregates were assumed to bind more strongly to the column.

2.2.4. Cationic ion exchange chromatography

Antibodies have a relatively high isoelectric point that means they are protonated and have a positive charge at not to low pH values. For that reason cationic ion exchange is (CIEX) normally used in bind-elute mode.

In industrial antibody manufacture processes, cationic ion exchange is a commonly used method as a second purification step following protein A affinity chromatography [12].

In order to enhance the elution, salt gradients are the most commonly used, but elution by means of pH gradients are also possible, or even a combination of both.

In this project the process flow was driven through a cationic ion exchange column after an affinity chromatography column and a size exclusion column to settle the pH.

2.2.5. Hydrophobic interaction chromatography

As opposite to the other chromatographic methods based on adsorption, hydrophobic interaction chromatography (HIC) is entropically driven [12] and is used to separate the antibody from its aggregate forms based on hydrophobicity as the aggregate forms are more hydrophobic. This method is not used in the setup studied at this work as an AIEX column is used as a final step to remove the aggregates instead of HIC column.

2.2.6. Affinity chromatography

Affinity chromatography separates proteins on the basis of interaction between proteins and a specific ligand linked to the chromatographic matrix. It can be used as long as a suitable ligand is available for the protein.

The target protein is bound to a complementary substance, the ligand. The material which is not bound is washed away and once that is done the conditions are changed to enhance

desorption, by changes in pH, ionic strength or polarity. In this project, desorption is enhanced by a pH reduction.

In order to purify IgG both Protein A and Protein G based columns can be used as IgG is able to bind to both, in this Protein A affinity column was modelled as it is the most widely used.

However this method is not effective when separating antibodies from its aggregates or fragments containing the Fab region.

2.3. Proposed purification setup

The setup studied consisted of a series of chromatography columns, in this setup the process flow is run continuously from a column to the next which means that the product from a column is the fed into the next column without an intermediate mixing step.

The purification consists of five steps; all of them based on chromatographic methods, and can be seen in the following picture.

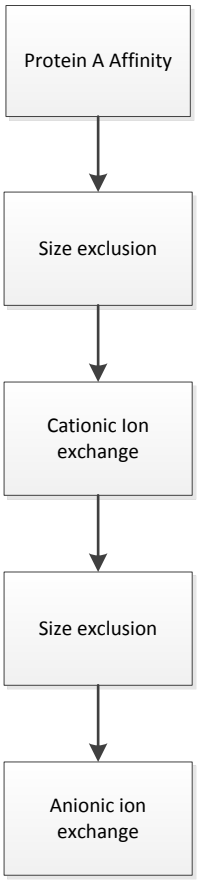

Figure 2 Process setup

The first step is an affinity chromatography column; this column is feed with the fresh feed containing the solution of IgG. It is assumed to be previously clarified so there are not cell debris and lipids present.

The column is expected to efficiently separate the IgG from impurities but not from the aggregates. It runs in bind-elute mode which means that the feed is loaded with binding conditions i.e. pH 5 and then the eluting is driven by decreasing the pH which leads to worse binding of the molecules to the column and the elution of these.

The affinity chromatography column is followed by a size exclusion column, the purpose of which is to stabilize the pH so that the following cationic ion exchange column is not fed with an acidic pH gradient. This is important since the binding on the molecules to the cationic ion exchange column depends not only on the salt concentration but also on the pH.

In normal manufacturing processes a virus deactivation step follows the affinity chromatography. In the virus deactivation the process flow is held during residence times longer than an hour at acidic conditions (pH~3). This virus simulation step can be implemented in the size exclusion step as long as the residence time at low pH is equal to at least one hour.

The third step is a cationic ion exchange column; this column further increases the separation of the components, allowing higher purity of the product IgG or better yield. This column also runs in bind-elute mode which means that the feed from the previous column first to bind to the column, and then start eluding when a salt gradient is applied.

The forth step is another size exclusion column the purpose of which is to reduce the salt concentration at the outlet of the cationic ion exchange. This is done by means of a size exclusion column as the salt ions are expected to elute later than the product due to their much smaller particle size.

The last step is an anionic ion exchange column running in flow-through mode where the aggregates and impurities are expected to bind to the column and elute later than the product.

3. Theory and mathematical modelling

There were five components modelled in this project, these are:

- IgG: The monoclonal antibody to be purified
- Aggregates: Dimers and other oligomers formed by IgG aggregation. Note that effect of aggregation during the purification process was not modelled, instead an initial quantity of aggregates was assumed to be loaded at the start of the purification process that had to be removed.
- Other impurities: The other impurities present, were modelled as a single component.
- Na^+: Salt ions that form the salt buffer used in the CIEX column.
- H^+: pH was modelled as the concentration of H^+ ions

All the mathematical models were based on the dispersion model for packed beds and are in the form of:

$$\frac{\partial C}{\partial t} = D_{axp}\frac{\partial^2 C}{\partial z^2} - \frac{v}{\varepsilon_T}\frac{\partial C}{\partial z} - \frac{1-\varepsilon_T}{\varepsilon_T}\frac{\partial q}{\partial t} \qquad (1)$$

Equation (1) takes into account the effect of dispersion, convection and adsorption. The adsorption term and the convection term are calculated differently for each column. But the dispersion is calculated the same way in all the cases.

This dispersion term corresponds to:

$$D_{axp}\frac{\partial^2 C}{\partial z^2} \qquad (2)$$

In order to include this dispersion term into the simulation the apparent dispersion coefficient must be known. This was calculated based on

$$Daxp = \frac{v \cdot L}{Pe} \qquad (3)$$

The characteristic length L in a packed bed is the particle diameter, Peclet number was assumed to be Pe~0.35 for all the columns.

Therefore apparent axial dispersion could be calculated as equation 4 shows:

$$Daxp = \frac{v \cdot d_{particles}}{0.35} \qquad (4)$$

The total porosity ε_T is defined as

$$\varepsilon_T = \varepsilon_c + (1 - \varepsilon_c) \cdot \varepsilon_P \qquad (5)$$

Where ε_c is the column porosity and ε_P is the packing porosity.

3.1. Affinity Chromatography

The affinity chromatography column separates the target IgG from the impurities based on their affinity to protein A. The binding of IgG to the chromatographic column is modified by the pH value.

This method is not effective in order to separate the antibody from its aggregates, as both are expected to bind similarly to the column. Also the low pH inside the affinity column might enhance the effect of aggregation however this effect is not modelled.

3.1.1. Affinity Chromatography Model

In order to simulate the affinity chromatography column a transport-dispersive model proposed by Candy K.S and others [13] was used. This model is a lumped parameter model that considers diffusion which is often the rate-limiting step in protein separation [14, 15].

The model used is a dispersion model that assumes homogeneity in the radial axis, isothermal adsorption and lumped coefficients for axial dispersion and mass transfer resistances [16].

The employed kinetic equation for the column can be described as:

$$\frac{\partial C}{\partial t} = D_{axp}\frac{\partial^2 C}{\partial z^2} - \frac{v}{\varepsilon_T}\frac{\partial C}{\partial z} - \frac{1-\varepsilon_T}{\varepsilon_T}\frac{\partial q}{\partial t} \quad (6)$$

$$\frac{\partial q}{\partial t} = k_m(q^* - q) \quad (7)$$

The mass transfer coefficient was to be calculated following the empirical correlation:

$$k_m = k_{max}\left[S_1 + (1 - S_1)\left(1 - \frac{q_R}{q_{max,R}}\right)^{S_2}\right] \quad (8)$$

In order to calculate the absorption isotherm two different approaches were taken into consideration.

For the target component IgG and the aggregates, it was defined as a Langmuir adsorption isotherm with pH as a modifier:

$$q^*_{igG} = \frac{q_{max}\cdot K_A\cdot\left(\frac{pH}{pH_{ref}}\right)^n C_{IgG}}{1+K_A\cdot\left(\frac{pH}{pH_{ref}}\right)^n C_{IgG}} \quad (9)$$

However for the impurities a linear adsorption isotherm is considered instead:

$$q^*_{imp} = H \cdot C_{imp} \quad (10)$$

The boundary conditions were defined as a no-flux boundary condition on the right end eq.(11) of the column and a Dirichlet condition on the other end eq.(11) where the concentration in that boundary is set to the concentration of the feed to the column.

$$C|_{Z=0} = C_{feed} \quad (11)$$

$$\left.\frac{\partial C}{\partial t}\right|_{Z=L} = 0 \quad (12)$$

The initial conditions for the column defined as a solute concentration of 0 and a pH of 5 which is also the pH of the load.

3.1.2. Affinity Chromatography Parameters

The physical parameters of the column to be simulated were set to match the manufacturer specification of "*HiTrap MAb Select SuRe*" columns [16].

Therefore the average diameter of the particles d_p was set to 85μm, the column length and column diameter were also set to 25mm and 7mm respectively.

The required empirical parameters for the model were taken from Candy K.S, and others article [13].

Table 1 Affinity chromatography parameters

Parameter	Symbol	Units	Value
Total porosity	ε_T		0.8
Maximum binding capacity	q_{max}	$g \cdot L^{-1}$	73
Association equilibrium constant	K_A	$L \cdot g^{-1}$	6.1
Linear isotherm constant	H		1.6
Maximum lumped mass transfer coefficient	k_{max}	s^{-1}	1.6
Saturation dependent kinetic constant	S_1		0.26
Saturation dependent kinetic order	S_2		4
pH dependent equilibrium order	n		16.6

The AC column is the first column in the purification process, and therefore the only column where fresh feed was simulated. The running conditions for the simulation were based on Thomas Müller-Spath and Massimo Morbidelli article [7]:

The feed into this column consist of 0.1 column volumes of a solution with the following characteristics:

- 0.4 g/L IgG
- 0.1 g/L Aggregates
- 0.1 g/L Other impurities
- pH 5
- 0 g/L of Na^+

The flow velocity in the column was set to 300 cm/h the concentration of IgG in the feed was set to 0.4 g/L the amount of feed loaded into the column was defined as 0.1 CV and the pH gradient during the elution was set from 5 after the load to 3 on a linear function over 300 seconds.

3.2. Size exclusion chromatography

In this work, size exclusion columns were employed in the purification process to stabilize and increase the pH from the AC column to the CIEX column and to reduce the salt concentration after the CIEX.

3.2.1. Size Exclusion Chromatography Model

The size exclusion columns were also modelled by means of a dispersion model. The main difference between the size exclusion column and the other kinds modelled (IEX and AC) is that there is no adsorption occurring in the column. This means that the separation is purely based on the total porosity of the column for each component and rate of diffusion into the particles.

Since the model used in this project for the size exclusion is dispersion model that means homogeneous concentration in the radial axis is assumed. The dispersion equation describing the model follows:

$$\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial z^2} - \frac{v}{\varepsilon_T} \frac{\partial C}{\partial z} \tag{13}$$

It's important to note that in this model that even though the column void is the same for all components the total porosity is different for each one as the packing porosity is also varying. This is done in order to reflect that the smaller particles can also get inside the particles whereas the bigger particles cannot.

Similarly to the affinity chromatography the boundary conditions for the model are set to no-flux (Von Neuman) to the right end, and Dirichlet condition on the left end, so the concentration of each component on the boundary are set to the values of the feed.

$$C|_{Z=0} = C_{feed} \tag{14}$$

$$\left.\frac{\partial C}{\partial t}\right|_{Z=Z} = 0 \tag{15}$$

The initial conditions are set as an absence of solutes and a pH of 7, which is inside the working range for this kind of column [3-12].

3.2.2. Size Exclusion Chromatography Parameters

For this column the physical parameters are set to match those of *"Superdex 200 Increase 10/300 GL"* specified by the manufacturer [18]. That is a column length of 300mm, a column diameter of 16mm and an averaged particle diameter of 8.6μm.

The void volume of the column was assumed as 0.3 of the total volume. And the packing porosity was also set to different values for each component.

The packing porosity for the IgG was assumed to 0.2 since it has a molecular weight of 150kDa it should not be able to diffuse properly into the pores.

For the first impurity considered which the molecules are smaller than IgG this value was estimated to 0.6.

The third impurity was meant as the molecules bigger than IgG such as dimer and other oligomers formed as a result of the aggregation, for that case the packing porosity was set to 0.15 as they shouldn't be able to enter the particles properly.

Lastly the packing porosity for the Na^+ and H^+ ions was set to 1 as these are expected to small enough to diffuse into all the pores present in the particles of this column.

The flow rate speed was set to the optimum flow rate defined by the manufacturer [18] of 57cm/hr.

3.3. Ion Exchange

Ion exchange chromatography is a method that allows the separation of components based on ionic interactions. This technique of separation can be used as long as we are treating with charged particles.

Due to the low and high pI of IgG respective forms this method of purification can be used as IgG does require neither too low pH values to be positively charged nor too high pH values to be negatively or positively charged.

3.3.1. Cationic Ion Exchange

The anionic exchange column was modelled by means of a dispersion model. The proposed model is a lumped kinetic model [19]. The kinetic equation for the column follows:

$$\frac{\partial C}{\partial t} = D_{axp}\frac{\partial^2 C}{\partial z^2} - \frac{v}{\varepsilon_T}\frac{\partial C}{\partial z} - \frac{1-\varepsilon_T}{\varepsilon_T}\frac{\partial q}{\partial t} \quad (16)$$

A linear driving force is assumed for the mass transfer i.e. a constant lumped mass transfer coefficient "k_m" is used in the equation that describes the adsorption.

$$\frac{\partial q}{\partial t} = k_m(q_i^* - q_i) \quad (17)$$

The adsorption equilibrium curve is defined by competitive Langmuir isotherm.

$$q^* = \frac{H_i \cdot C_i}{1+\sum_{i=1}^{n}\frac{H_i}{q_{sat,i}}\cdot C_i} \quad (18)$$

The Henry coefficients are defined as a power function based on the salt concentration [19].

$$H_i = \alpha_{1,i} \cdot c_{salt}^{\alpha_{2,i}} \quad (19)$$

As in the other columns the boundary conditions were defined as a no-flux boundary condition on the right end of the column and a Dirichlet condition on the other end where the concentration in that boundary is set to the concentration of the feed to the column.

$$C|_{Z=0} = C_{feed} \quad (20)$$

$$\left.\frac{\partial C}{\partial t}\right|_{Z=Z} = 0 \quad (21)$$

The initial conditions for the column defined as an initial concentration of Na^+ of 0.0025 mol L^{-1} and a pH of 6.

3.3.2. Cationic Ion Exchange Parameters

The column physical parameters were taken from the data provided by the manufacturer of *"POROS HS 50"* columns [21]. That is a column length of 10cm, a column diameter of 1.2 cm and an average particle diameter of 50μm.

The porosity, mass transfer and Henry coefficients found by T.Müller-Späth et al. [19] are set as:

Table 2 CIEX paramters

Parameter	Symbol	Units	Value
Porosity for IgG and impurities	ε_T		0.58
Mass transfer coefficient	k_M	min^{-1}	3.3
Saturation capacity	q_{sat}	$g \cdot L^{-1}$	110

Table 3 CIEX Henry coefficients

Component	α_1	α_2
IgG	94708	-6.34
Impurity 1	312000	-6.34
Impurity 2	6660	-6.34
NaCl	2	0

The concentration of salt in the load was defined as 0.0025 mol L^{-1} after the load is finished the salt gradient starts, that is linear increase from 0.0025 to 0.25 mol L^{-1} over 600 seconds.

The flow rate of the column was set to 300cm/hr.

3.3.3. Anionic ion exchange

The anionic exchange column was modelled by means of a dispersion model. As we had no information about the adsorption occurring in the column, the model was simplified to that of a size exclusion column i.e. no adsorption effects are being considered.

$$\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial z^2} - \frac{v}{\varepsilon_T}\frac{\partial C}{\partial z} \qquad (22)$$

The boundary and initial conditions are defined as in the other size exclusion column columns.

$$C|_{Z=0} = C_{feed} \qquad (23)$$

$$\left.\frac{\partial C}{\partial t}\right|_{Z=Z} = 0 \qquad (24)$$

The initial conditions for the column defined as an absence of solutes and a pH of 6.

3.3.4. Anionic Ion Exchange Parameters

The column physical parameters are the same that the ones used for the size exclusion column but the packing porosity is set to lower for the IgG, Na^+, H^+ and higher for the impurities, this is to represent the effect of the impurities binding to the column while the IgG is supposed to flow through.

The difference in a flow through simulation is that since the product leaves first the entire outlet of the column is collected as part of the pool until the concentration of impurities reaches a certain value.

3.4. Alternative models

An interesting alternative to the mentioned method would be to implement heterogeneous model for packed beds which would take into account the effect of diffusion into the particles.

$$\frac{\partial C}{\partial t} = D_{ax}\frac{\partial^2 C}{\partial z^2} - \frac{v}{\varepsilon_c}\frac{\partial C}{\partial z} - \frac{1-\varepsilon_c}{\varepsilon_c}\frac{3}{R_p}k\left(C - C_p\big|_{r=Rp}\right) \quad (25)$$

$$\frac{\partial C_p}{\partial t} = \frac{D}{\varepsilon_p}\left(\frac{\partial^2 C_p}{\partial r^2} + \frac{2}{r}\frac{\partial C_p}{\partial r}\right) \quad (26)$$

Note that this model uses dispersion coefficient instead of the apparent dispersion coefficient, and packing porosity instead of total porosity.

With the following boundary conditions:

$$C|_{Z=0} = C_{feed} \quad (27)$$

$$\left.\frac{\partial C}{\partial t}\right|_{Z=Z} = 0 \quad (28)$$

$$\left.\frac{\partial C_p}{\partial r}\right|_{r=0} = 0 \quad (29)$$

$$\left.\frac{\partial C_p}{\partial r}\right|_{r=Rp} = k\left(C - C_p\big|_{r=Rp}\right) \quad (30)$$

This model offers a more realistic description of the process in the packed bed and would be especially interesting to implement as an alternative to the current SEC model since in the SEC there is no adsorption happening and the separation is based only on the different rate of diffusion into the column particles.

However model adds complexity and drastically increases calculation times since the total number of mesh points employed in the finite volume method is equal to the number of mesh points in the column multiplied by the number of mesh points in the particles.

3.5. Connections between columns

3.5.1. Pool definition

After each column only a part of the outflow is taken and loaded into the next column while the rest of the outflow is thrown away. The part that is to be loaded into the next column is the pool. Since the different steps of the setup have different purposes three different pool types were defined, all of them based on Niklas Andersson simplexpooling function.

3.5.1.1. Pool based on main component purity

This is the pool used for affinity chromatography and Ion exchange steps. Here a required purity of the objective component IgG is set. The pool is the larger amount of outflow that can fulfil that purity requirement.

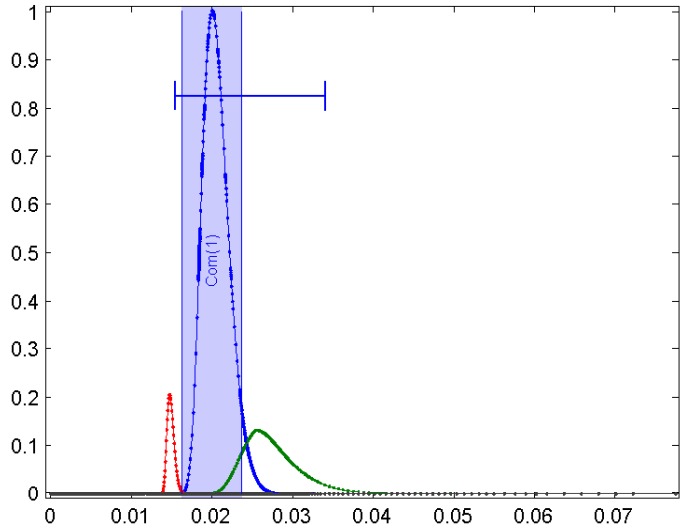

Figure 3 Purity pool example

3.5.1.2. Pool based on pH

After the affinity chromatography step the outflow has a very acidic pH (around 3), therefore a size exclusion column follows previous to the ion exchange step. The main purpose of this column is to set the pH value to that required by the following Cationic Ion exchange column, which is pH 6.

The cationic ion exchange is dependent on the pH on the pool region should also be stable.

After this step a pool is performed with the objective of obtaining the desired pH, the impurity for this pool is defined as the absolute value of the difference between the concentration of H^+ ions and the concentration of H^+ ions at pH 6.

$$pool_{imp} = |C_{H^+} - 10^{-6}| \quad (31)$$

3.5.1.3. Pool based on salt concentration

Similarly to the previous after the ion exchange column another size exclusion column follows in order to reduce the salt content. In this type the impurity is defined as the salt content.

3.5.2. Load definition

As explained before the setup to be studied consists of a series of chromatography columns.

The initial feed flow goes into the first column and then a pool of the outflow of this first column is fed into the second column continuously. This process is repeated until the last column.

As a result of this the inflow or into all the columns (excluding the first one), will have the shape of different "peaks" for each component as a result of the chromatographic separation in the previous column. Figure 4 shows the outflow of the first AC column as an example.

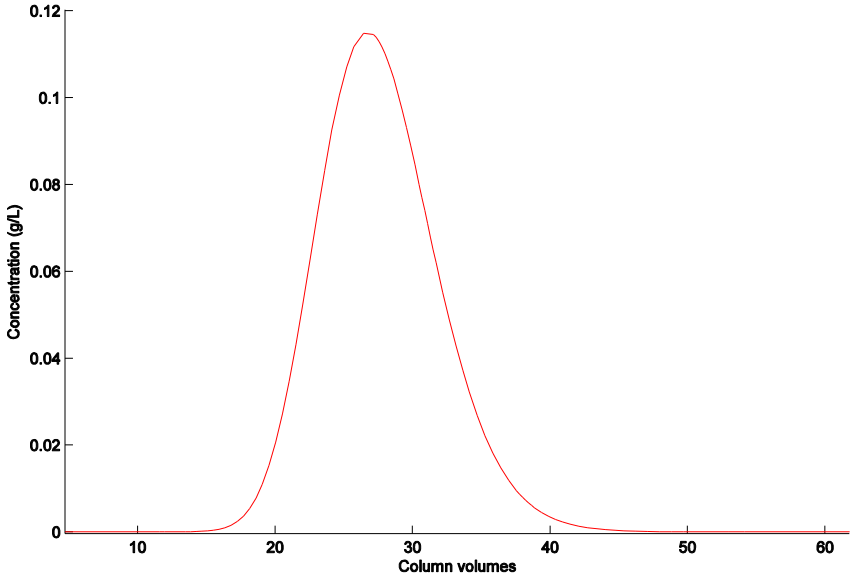

Figure 4 AC IgG outflow

In Matlab environment the data of each component outflow comes in the form of discretised concentration values of each component for each time value.

Since this data was to be used in the simulation of the following column it had to be interpolated into a continuous function that was named load function.

For this problem four different interpolation methods were studied, those were: Polynomial interpolation, Piecewise linear interpolation (Linear spline), Piecewise polynomial interpolation (cubic spline) and Gaussian functions with multiple terms.

3.5.2.1. Polynomial Interpolation:

This is a well-known method of interpolation. It consists of interpolating the data to a function of the form of:

$$f(x) = a_0 + a_1 x^1 + a_2 x^2 + \cdots + a_n x^n \qquad (32)$$

The higher the n value is, the better fit is to be expected, but this will also increase calculations time, therefore it's recommended to keep this value as low as possible.

The generic curve show before was interpolated for the main component using polynomial interpolations with different "n" values.

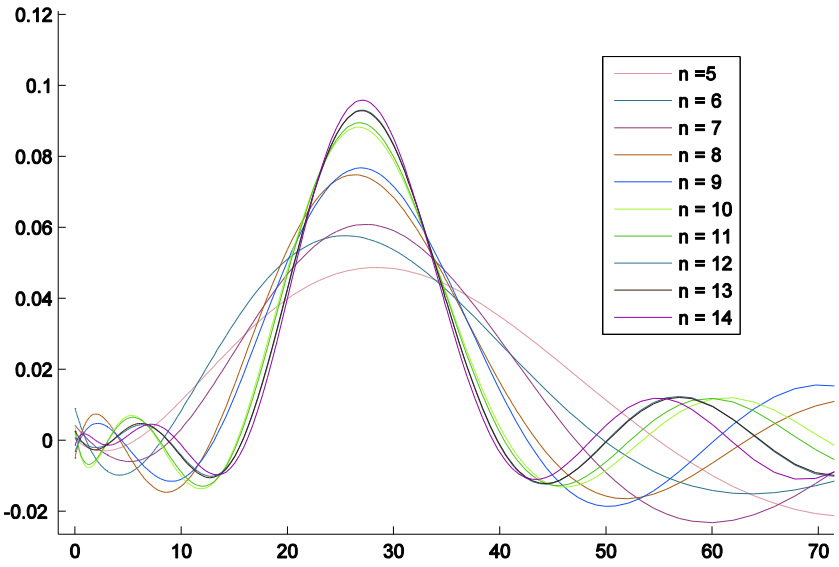

Figure 5 Polynomial interpolation

The figure shows the interpolation with different grade equations. It can be observed that lower grade polynomial interpolation offer a bad fitting but higher grade polynomial interpolation also show a strong effect of Runge's phenomenon, that is oscillation at the edges of an interval.

3.5.2.2. Linear spline

One way to avoid this Runge's phenomenon is the use of spline interpolation. Spline interpolation uses a function for each time interval. The simplest case of spline interpolation is linear spline interpolation. It has the form of:

$$f(x) = a_{00} + a_{01}x \quad if\ x \in [x_0, x_1]$$

$$f(x) = a_{10} + a_{11}x \quad if\ x \in [x_1, x_2]$$

...

$$f(x) = a_{n0} + a_{n1}x \quad if\ x \in [x_n, x_{n+1}] \qquad (33)$$

When using this kind of interpolation a better fit is obtained:

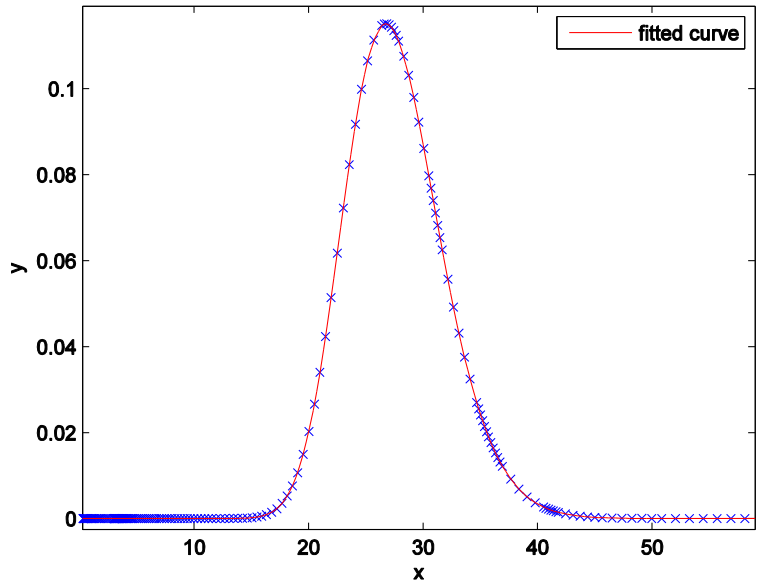

Figure 6 Linear interpolation

Even though the R^2 of this kind of interpolation is the maximum R^2=1 this is not to be interpreted as an indicator of the "goodness" of the fitting since by definition spline is forced to pass through all the data points but the fit is not perfect as it can be seen with a more on detail figure.

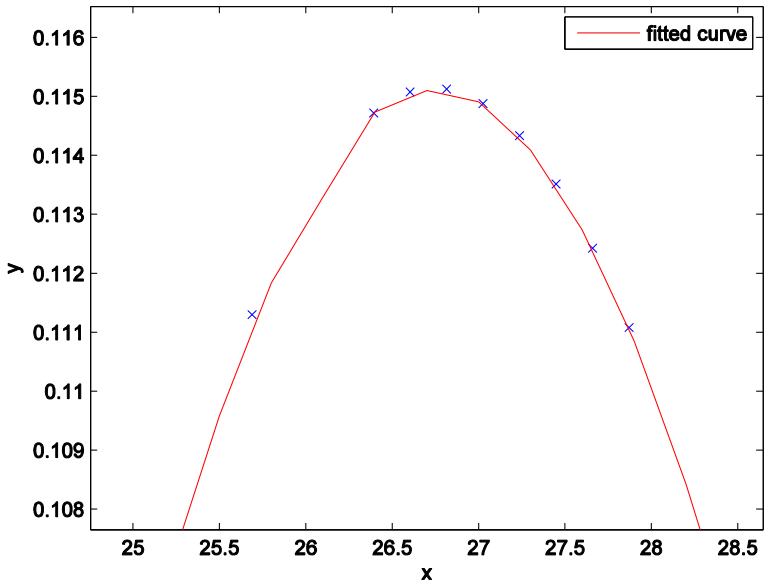

Figure 7 Lspline detail

As it can be seen the fit is not ideal since the area below the curve which is a crucial parameter (the total amount of component) might not be properly defined.

3.5.2.3. Cubic spline

This interpolation is similar to the linear spline; it is based on the same idea i.e. using different functions for each segment but in this case. This offers a best fit than linear spline

but it is also unaffected by Runge's phenomenon and it is still faster to calculate than very high degree polynomial interpolations.

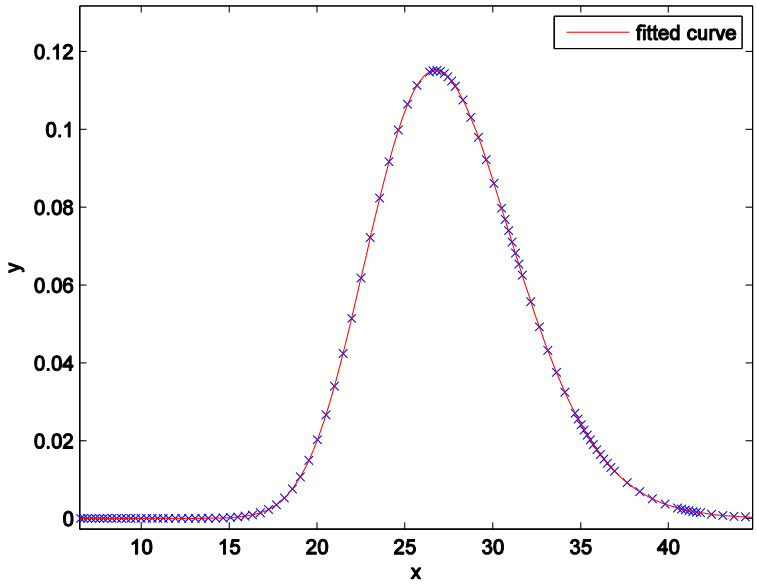

Figure 8 Cubic spline

This method used functions of the form:

$$f(x) = a_{i0} + a_{i1}x + a_{i2}x^2 + a_{i3}x^3 \quad if\ x \in [x_n, x_{n+1}] \qquad (34)$$

In the detail view it can be see that this kind of interpolation offers a curve that bends to the point giving a smoother and better fit. See figure 9

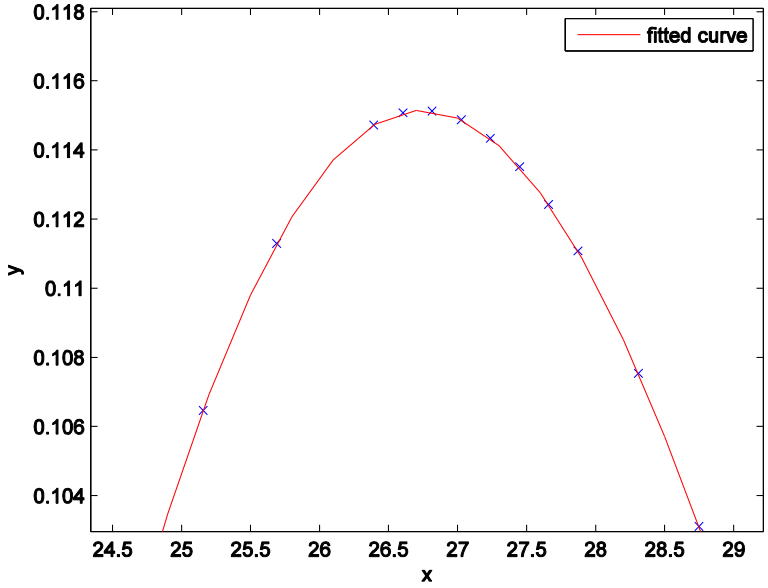

Figure 9 Cubic spline detail

3.5.2.4. Gaussian function

The last method of interpolation which was tested was using Gaussian functions with multiple terms. The Gaussian curves might give a good fit to the peaks as long as they are symmetric. The advantages of using a Gaussian curve is that it helps to mitigate the effects of noise and are useful to predict the curve evolution if only a few points are known, but this offer no advantage in the current situation since the results of simulation are not affected by noise and do not require extrapolation.

A one term Gaussian function has the form of:

$$f(x) = a \exp\left(-\frac{(x-b)^2}{2c^2}\right) \tag{35}$$

The result can be seen in the following figure:

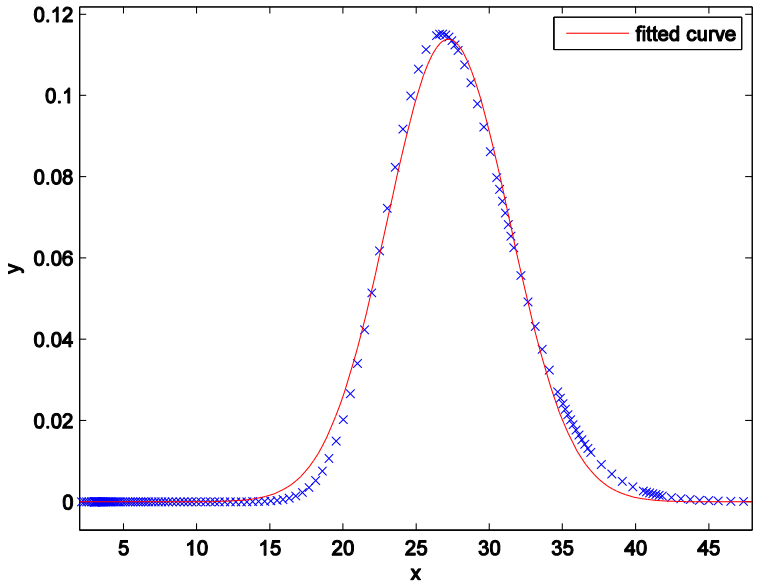

Figure 10 Gaussian function

It can be seen that a bad fit is obtained with a R^2 of 0.975

A fit to three term Gaussian function was also performed, which has the form of:

$$f(x) = a_1 \exp\left(-\frac{(x-b_1)^2}{2c_1^2}\right) + a_2 \exp\left(-\frac{(x-b_2)^2}{2c_2^2}\right) + a_3 \exp\left(-\frac{(x-b_3)^2}{2c_3^2}\right) \tag{36}$$

The result is:

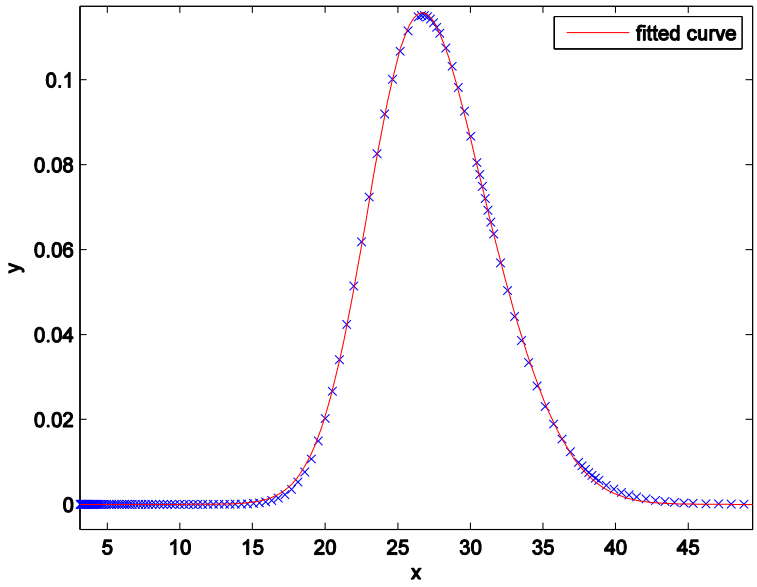

Figure 11 Three term Gaussian function

It can be observed that the fit is significantly better and the R^2 of this example curve can be calculated to 0.996

3.5.2.5. Implementation

After the evaluation of the different interpolation methods it was decided to adopt the cubic spline as an interpolation method for the load function definition. This method was chosen since it does not suffer the same oscillation effect than a polynomial interpolation and it offers better fit than a linear spline, and a Gaussian fit. Also the benefits of employing a Gaussian curve (noise reduction and better extrapolation) are not expected to be useful in this case.

4. Results and discussion

All the different models were solved using the finite volume and Matlab® ode15s function. Also a three point central approximation and a two point backward approximation were used for the first and second order derivatives discretization respectively.

4.1. First step (AC)

As stated before, the first step in the purification and hence the first step in the calculation process is an affinity column. At this step is expected to provide good separation of the IgG from the small impurities but not from the aggregates.

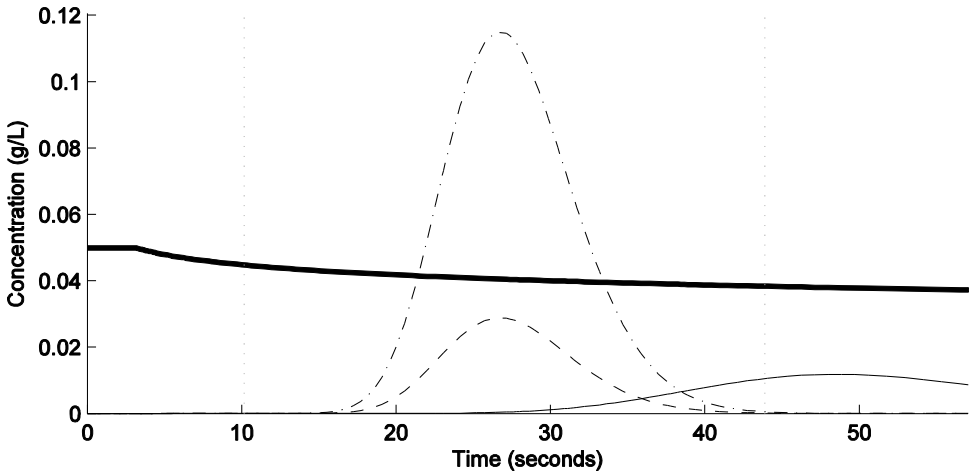

Figure 22 AC chromatogram

As the aggregates are not at separated from IgG the pooling on this step is based on the amount of other impurities. This are set to a maximum of 0.05 the concentration of IgG in the pool. That produces a pool with the following concentrations (if it were to be homogeneously mixed).

Table 4 AC pool concentration

	Concentration g/L
IgG	0.0356
Aggregates	0.0089
Other impurities	0.0023

As expected this step provides very good separation of IgG from a range of the impurities, while keeping IgG productivity high. The IgG yield from this step was calculated to 0.9992.

In order to plot everything in the same plot the pH value was scaled down 100 times, a closer look can show how the pH gradient enhances the elution of the different components.

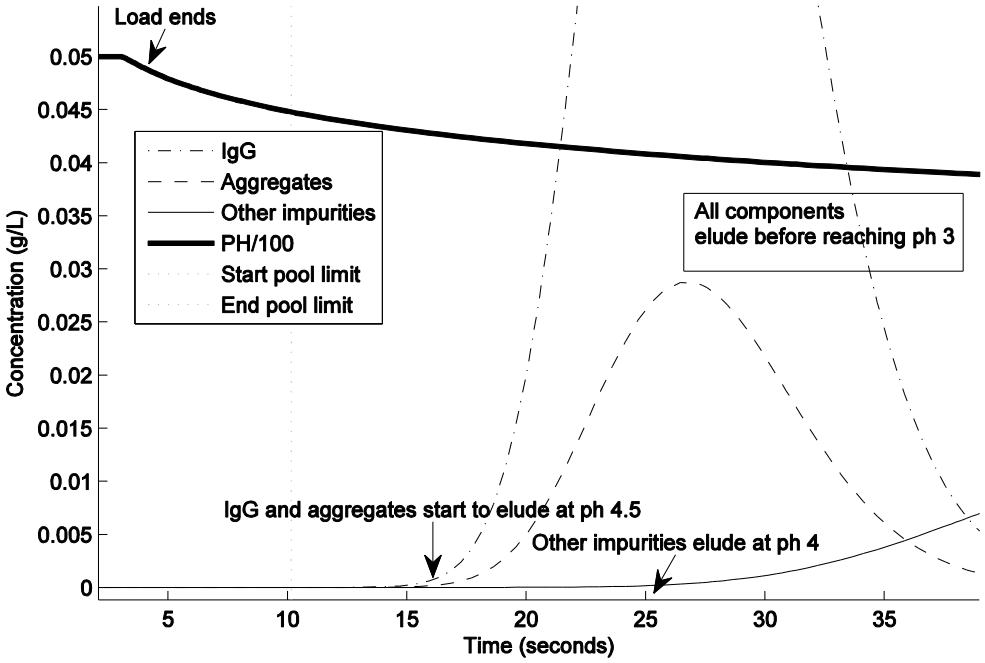

Figure 13 AC chromatogram detail

4.2. Second step (SEC)

This step is included to stabilize the pH of the solution that is to be loaded to the next column. As it could be seen from previous column, the pool obtained from this column has a pH in the range from 5 to 4. That load would provide bad conditions for the CIEX column as the pH would affect the binding to the column.

The purpose of this column is to "displace" the pH gradient from the IgG peak. The column is initially loaded with a solution at pH 6 and the separation can be achieved through size exclusion chromatography as the H^+ are expected to elute later than the other components since they can go through more space in the column due to their much smaller size.

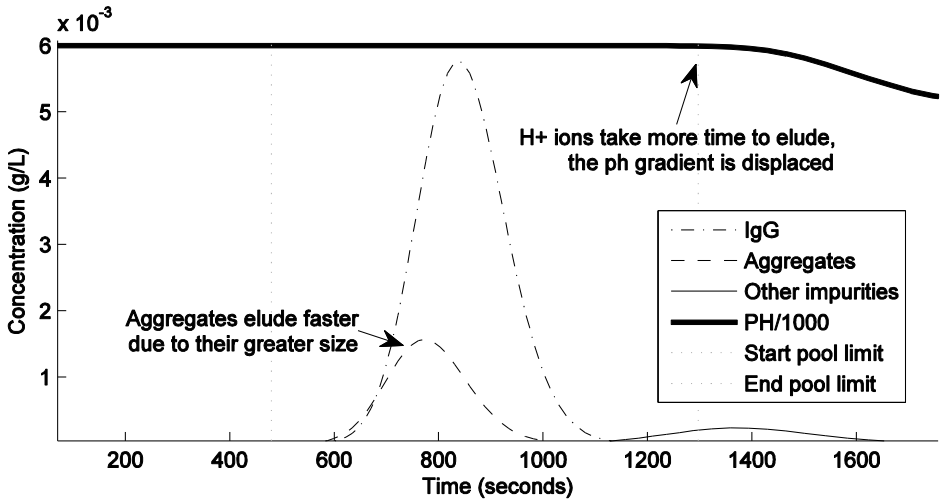

Figure 14 Size exclusion chromatogram

As it can be seen in the previous figure, the increase in H^+ concentration that represent a pH drop is displaced from the IgG concentration peak. This of course assumes that H^+ ions behave as larger molecules do in a size exclusion bed which might not be strictly true.

As a side effect the elution volume is greatly increase due to the length of the column (30cm) and the slow flow rate compared to the other chromatographic methods.

As the pH gradient has been significantly displaced the pool is capable of containing the totality of the IgG. This means that they yield of IgG in this purification step is ~1.

However the concentration of all the components drop equally, since the elution volume has increase largely as a result of the dispersion in method that uses a much larger column (30cm) with slower flow rate than the other chromatographic methods.

4.3. Third step (CIEX)

The cationic chromatography purification step follows. Here, IgG is expected to bind first at low salt buffer conditions and then elute with the salt gradient. The pH of the feed solution is now adequate, since a varying pH might induce variations in the binding of to the column.

First this was simulated using a salt concentration on the initial buffer of 0.05, which lead to improper separation of the different components:

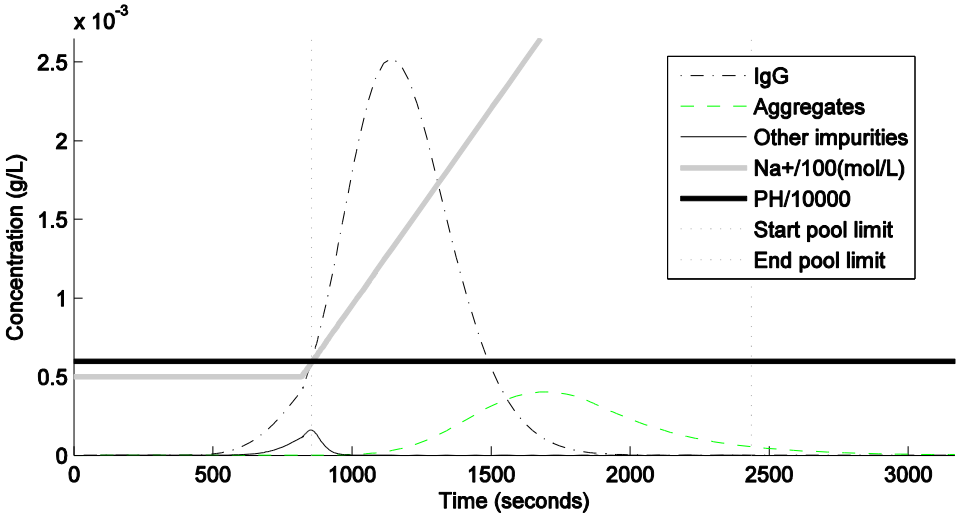

Figure 15 CIEX chromatogram 1

As it can be observed from fig 15 the high volume of load combined with relatively high initial buffer led to the components eluding even before the load had ended. This result in improper purification as the products elude at the same time.

In order to avoid so the simulation was set to more strongly binding conditions i.e. an initial salt buffer of 0.0025 mol/L Na^+.

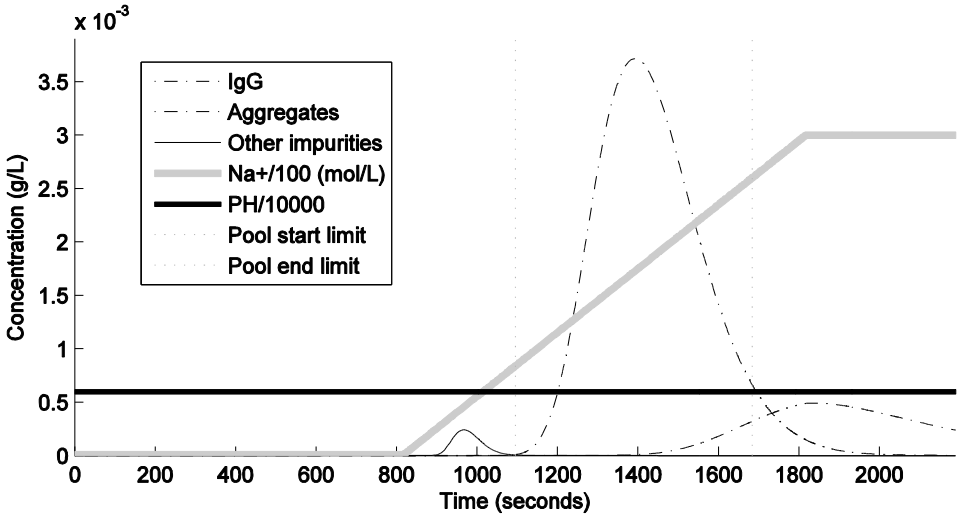

Figure 16 CIEX chromatogram 2

From this last figure it can be see that a high percentage of the aggregates can be pooled away, and almost the totality of the other impurities can be eliminated. The reason for the aggregates not being able to separate completely is probably due to the fact that these might be positively charged similarly to the IgG at pH 6 conditions.

An interesting effect of this purification step is that the aggregates and other impurities peaks switch their respective positions towards the IgG peak. In contrast to the other methods the aggregates now elute later whereas the other impurities elute earlier.

As the following columns (SEC, and AIEX in flow through mode) might displace the “other impurities” peak to the right (longer elution times than IgG), they are to be effectively removed in this step.

Therefore the pooling requirements for this step were defined as 0.97 purity of IgG, and a limit of 0.005 of the non-aggregate impurities. The obtained yield of IgG was 0.952 and the pool elution time was reduced 588 seconds.

4.4. Forth step (SEC)

As explained in the introduction the forth step is another size exclusion chromatography column but this one yields a different purpose. Instead of stabilizing the pH which is already 6, this column is meant to eliminate the salt content in the pool.

Similarly to the H^+ ions, Na^+ ions should be separated from the IgG in a size exclusion column.

However a negative effect from the SEC column is to be expected as the aggregates might have shorter residence time than the IgG, and therefore elute closer than they did in the previous CIEX step.

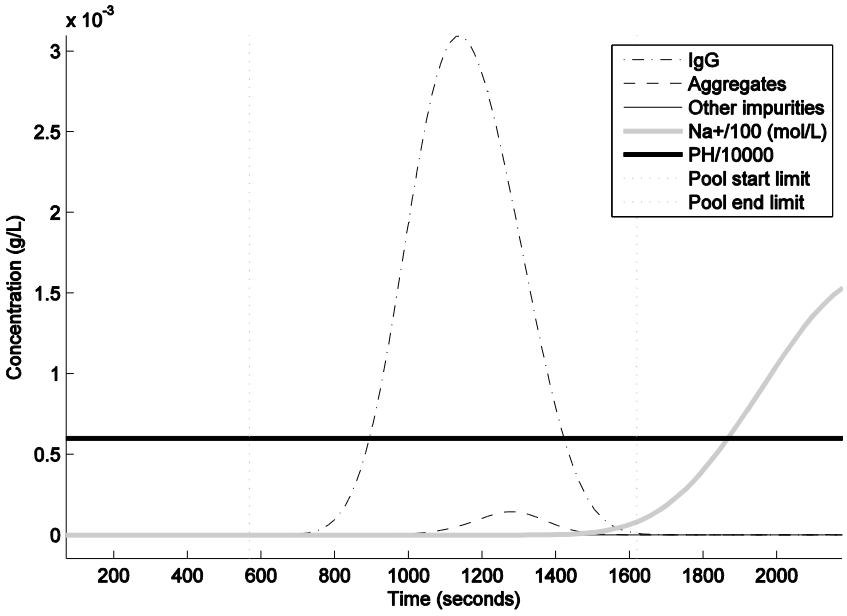

Figure 17 Second SEC column chromatogram

As expected the salt gradient can be effectively separated whereas the aggregates peak is slightly moved towards the centre of the IgG peak.

The pool for this step was defined so that the concentration of salt in the pool would be lower than $5.6 \cdot 10^{-4}$. The yield of IgG was 0.9993 and the elution time was equal to 1052 seconds.

4.5. Last step (AIEX)

The last step consists of an anionic ion exchange column running in flow through mode but as explained in the mathematical modelling section it is simplified to the model of a SEC, with modified parameters.

The idea behind the AIEX in flow through mode is that the IgG might elude first whereas the aggregates and impurities are retained.

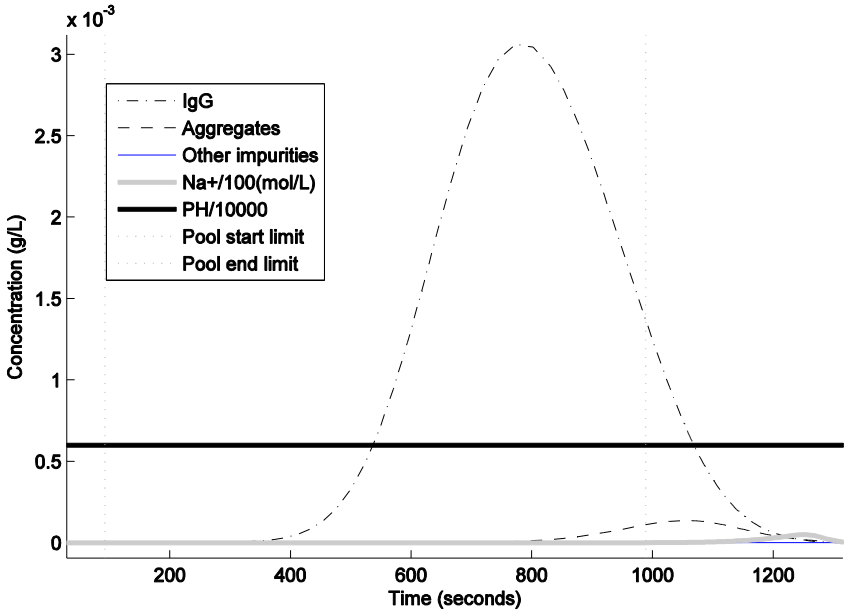

Figure 18 Anionic ion exchange chromatogram

This results in a reasonably good separation. The pool is performed so that the concentration of aggregates in the pool is 0.0001.

The defined pool elution time is 897 and the obtained yield of IgG is 0.9. The concentration of the different components in the pool is:

Table 5 Purification results

	Concentration g/L	% of total
IgG	0.0011	90.8%
Aggregates	0.0001	8.25%
Other impurities	0.0000115	0.95%
Salt	0.000001	0.01%

The yield of IgG produced from the entire purification process can be described as:

$$Yield_{total} = Yield_{Step1} \cdot Yield_{Step2} \cdot Yield_{Step3} \cdot Yield_{Step4} \cdot Yield_{Step5} = 0.856 \quad (37)$$

4.6. Further work

There are several points from where this project could be further developed, the most interesting option would be:

4.6.1. Process optimization

There are several parameters that could be optimized; first the pH and salt buffers and gradients could be optimized for either most yield or highest purity.

Also a multivariable optimization could be performed being the purity requirements on each step the variables and total yield of IgG the objective.

4.6.2. Adopting an heterogeneous model

As mentioned in the alternatives to the mathematical models, a general model could be useful to implement especially for the size exclusion chromatography, as it might provide more accurate results.

4.6.3. Develop the first SEC to resemble a virus deactivation step

It was mentioned that a virus deactivation step usually follows the affinity chromatography, that is because a virus deactivation step requires a long residence time~1h at low pH~3. This step is not modelled but the first size exclusion column could be modified to accomplish this requirements.

4.6.4. Scale up

The simulation is developed around lab-scale parameters from bibliography. The work could be scaled up to design an industrial production level purification.

4.6.5. Add mixing tanks in the process

In the current process there some steps where the position of the different concentration peaks change position. This happens in the steps before and after the cationic ion exchange column.

Adding a continuous stirred tank (CSTR) before the CIEX might increase the separation in this step on the other hand adding a CSTR after the CIEX might increase the separation in the following steps.

This might however increase the overall time consumed by the process so effect on productivity should also be studied.

4.6.6. Developing anion exchange model

The anion exchange column was modelled as a modified size exclusion column, since the effect of adsorption into this column was unknown. Developing and implementing an anionic exchange model would provide more accurate results.

4.6.7. Modelling aggregation effects

As it was previously mentioned, aggregate formation is not implemented in the simulation. An initial concentration of aggregates is assumed in the first load instead. It would be interesting to model the effect of aggregates in the different chromatographic columns as well as the effect during the pumping of product from one column to another.

5. Conclusion

- The purification process was successfully modelled and the results showed an IgG purity of 90.8% and yield of 0.856.
- Size exclusion columns proved an effective method to stabilize the pH gradient and remove the salt content.
- Some steps separate the components in different directions. In the first SEC column the aggregates elute faster than the IgG whereas in the following CIEX aggregates elute later. Adding a mixing step between the two columns instead of a continuous feed from one to the other is to be considered.
- The anionic ion exchange step provides a separation of IgG from its aggregates worse than it was expected. Implementing a more accurate model is probably required for better results.

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7. Appendices

Table of symbols

Latin symbols		
Symbol	**Definition**	**Unit**
C	Concentration	g/L
t	Time	s
D_{axp}	Apparent axial dispersion coefficient	cm^2/s
L	Characteristic Length	cm
k_m	Lumped mass transfer coefficient	1/s
q	Adsorbed solute	g/L
q*	Adsorbed solute at equilibrium	g/L
k_{max}	Maximum lumped mass transfer coefficient	1/s
S1	Saturation dependent kinetic constant	
S2	Saturation dependent kinetic order	
q_{max}	Maximum binding capacity	g/L
q_{maxR}	Maximum binding capacity of all the retained solutes	g/L
q_R	Sum of all the retained solutes concentrations in the stationary phase	g/L
q_{sat}	Saturation capacity	g/L
H	Henry coefficient	
K_A	Association equilibrium constant	L/g
z	Spatial dimension z	cm
n	Ph dependet equilibrium order	
Rp	Particle radius	cm
k	Mass transfer coefficient	cm/s
a_{nm}	Interpolation coefficients	
a,b,c	Gaussian interpolation coefficients	

Greek symbols		
Symbol	**Definition**	**Unit**
ν	Velocity	cm/s
ε	Porosity	
α_1	Empirical constant for Henry function	
α_2	Empirical constant for Henry function	