Optimization of a PEGylation process

A combined reaction and separation with Size Exclusion Reaction Chromatography

by

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Picture on front page: SERC-column version 2. Picture made by Fredrik Tegnér.

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Abstract

The PEGylation process is a covalent attachment between a protein (the pharmaceutical) and poly ethylene glycol (PEG) and since the beginning in 1977 PEGylation processes have been used to improve pharmaceuticals. PEGylation of a pharmaceutical achieves improved properties like greater solubility in water, longer residence time in vivo and extended shelf life.

The PEGylation process is in general conducted with a batch reactor connected to a size exclusion chromatography (SEC) column or more common an ion exchange chromatography (IEC) column. The batch reactor achieves a yield of monoPEGylated protein at approximately 60 % and a 10 % yield of multiPEGylated proteins. Other processes are still under development like the size exclusion reaction chromatography (SERC).

The report contains two parts, an experimental part and a simulation part. The experimental section tests the batch reactor in order to calibrate the kinetic constants. Experiments with a SERC column were also conducted. The simulation section created models for the batch reactor, the SEC column and the SERC column. The batch reactor model includes four reactions, three PEGylation reactions and one deactivation reaction. Both the SEC column and the SERC column are described with the General rate model. The SERC column is combined with a recirculation cycle and optimized for different objectives.

The experimental results show fast kinetic reactions for the PEGylation that is suitable for the SERC column. The SERC column experiments resulted in a selective monoPEGylated protein production. The simulations resulted in a monoPEGylated protein yield at 82.3 % when recirculating the unPEGylated protein nine times.

In future research a more detailed recirculation cycle can be simulated and validated with experiments. Also an automated injection loop where the reactants are mixed when entering the SERC column is able to improve the results.

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1 Introduction

1.1 Background

The PEGylation research started in 1977 by Abuchowski et al. (1). The research showed that covalent attachment between poly ethylene glycol and bovine liver catalase improved multiple properties of the native protein, such as greater solubility in water, longer residence time in vivo and extends shelf life. (1,2)

In 1990, the first PEGylated product reached clinical practice, PEG-adenosine deaminase (Adagen®) against severe combined immunodeficiency disease (SCID). Between 1990 and 2011 a total of ten PEGylated products were approved for clinical practice fighting diseases such as Leukemia and Hepatitis C. The number of approved PEGylated pharmaceuticals will increase due to high interest among pharmaceutical companies. (3)

A major disadvantage with the PEGylation process is the many mixtures of PEGylated proteins given in the reaction. The PEG polymers often have the possibility to attach to the protein in multiple free sites resulting in multiPEGylated proteins. This means that after a batch reaction process a number of different PEGylated proteins plus the native protein will exist together with the product. Although these different types of PEGylated proteins are easy to separate, a large amount of native protein will still go to waste in the process. (2,4)

Today the PEGylation process is carried out with a batch reactor and the products are separated with either an ion exchange chromatography or a size exclusion chromatography. There is a lot of research to improve the yield and selectivity, and one of the new ideas are to separate the reactants for the product before the products can PEGylate further. This can be done in a size exclusion reaction chromatography column. (5)

1.2 Aim

The report contains two parts, an experimental and a simulated. The experimental chapter will focus on reproducing the batch PEGylation reaction of Maiser et al. (6) using mPEG-propionaldehyde and Lysozyme in purpose of finding a reaction model. The experimental chapter will also include experiments with combine reaction and separation in a size exclusion chromatography column.

The simulation section will start with calibration of the kinetic constants and the porosity constants from the experimental results. Three models are constructed, one batch reactor model, one size exclusion chromatography column model and one size exclusion reaction chromatography column model. The models will be validated against the experimental data. The SERC model is then used in an optimization problem where different objectives are calculated.

The hypothesis of the report is that recirculation of Lysozyme combined with the size exclusion reaction chromatography column will give a highly selective monoPEGylated protein reaction without lowering the yield.

2 Theory

2.1 PEG-chain

Poly ethylene glycol (PEG) is a neutral polymer, which in its most common configuration (Figure 2.1) is a linear polyether that is terminated with a hydroxyl group in each end. PEG has a high solubility in both aqueous and organic solvents which makes it a highly versatile molecule in biological chemistry. Other important properties are that the polymer is non-toxic, non-immunogenic and easy to control the molecular weight and applying a large range of functional groups. (7–10) Because of the high solubility PEG is able to take up a large amount of water giving the molecule a larger viscosity radius. This means that a molecule with a specified molecular weight in a size exclusion chromatography column is seen much larger. (2,8)

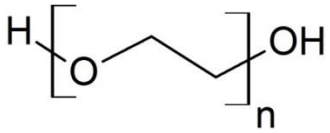

Figure 2.1: Poly ethylene glycol in the most common form where n is equal a positive integer.

In the PEG molecule's common state the molecule have a low reactivity against other molecules. In order to be able to use PEG in various reactions it must be activated with a functional group at one or both ends. The activation processes have been divided into two generations. In the first generation, PEG-chains are often affected with negative properties such as impurities, low molecular weights and unstable linkage. In PEGylation the first generation PEG chains are attached to either the alpha or epsilon amino groups for example PEG dichlorotriazine, PEG trichlorophenyl carbonate and PEG succinimidyl succinate (PEG-NHS seen in Figure 2.2). (7,11)

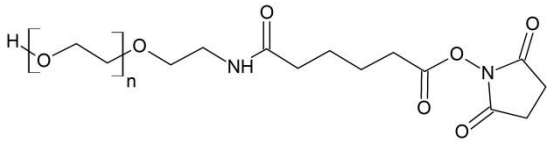

Figure 2.2: A typical first generation PEG chain. This molecule is called PEG-NHS.

In the second generation, PEG chains are produced to avoid the first generation inadequacies. One of the first examples is the first generation PEG chain PEG-acetaldehyde that was substituted with the second generation PEG chain mPEG-propionaldehyde (Figure 2.3). This exchange resulted in prevention of impurities by aldol condensation. Another improvement with the most second generation PEG chains is the extension of the chain giving an increase in half-life. (7,11)

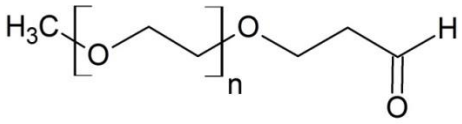

Figure 2.3: One of the first second generation PEG chains used in experiments. This molecule is also used in the experiments later in the report.

2.2 Reducing agent

A reducing agent is necessary in order to complete a PEGylation reaction between a protein and mPEG aldehydes. The reducing agent has an amount of requirements that must be fulfilled. One requirement is that the reducing agent needs to be selective and reduce the correct functional group. The most common reducing agent used in PEGylation processes is sodium cyanoborohydride, $NaCNBH_3$. (12,13)

Sodium cyanoborohydride is used in the PEGylation process because of a couple of properties. The major property is that sodium cyanoborohydride is effective in attaining a site specific PEGylation. Other properties are that sodium cyanoborohydride is highly soluble in aqueous solutions, tough and efficient reducing agent. The drawback with using sodium cyanoborohydride is that the reduction reaction produces very toxic hydrogen cyanide. (12,13)

Research to find a reducing agent with the same properties as sodium cyanoborohydride is largely conducted due to its highly toxic byproduct. Many types of borane class agents have been tested due to the borane molecules ability to selectively reduce specific functional groups. Similar results shows when PEGylating the Recombinant human interleukin 10 protein with the reducing agents pyridine borane and 2-picoline borane compared with results when PEGylating with sodium cyanoborohydride. (13)

2.3 Size exclusion chromatography

Size exclusion chromatography (SEC) separates molecules according to size. The separation can be achieved due to porous particles packed inside the column. Large molecules can only access the volume outside the particles (void volume) and small molecules can access both the void volume and the particle pores (Figure 2.4). Large molecules will therefore travel faster through the column and smaller molecules will follow according to size. (14)

The SEC columns possibility to separate molecules is determined by the particles pore size and column length. Large pore sizes (~100 nm) have the ability to separate molecules up to 1,500,000 Da and small pore sizes (~5 nm) have the ability to separate molecules up to 5000 Da. The closer the different molecules are according to size the longer the column is needed to get a satisfying separation. To be able to separate a wide range of molecule sizes a column packed with particles with different pore sizes is preferable. (14)

The SEC column is an excellent choice for the separation part of the PEGylation process. Due to the high molecular weight of the PEG chains it is possible to separate the different PEGylated proteins from each other and the native protein. Another advantage with the PEG chains combined with SEC is that PEG chains appear larger than its real molecular weight. As mentioned before PEG chains have the possibility to bind water and therefore achieve larger viscosity radiuses which in a SEC column will look like the PEG chain have a much larger molecular weight. This makes it possible to separate PEGylated proteins with high accuracy even though a small PEG chain is used. (15)

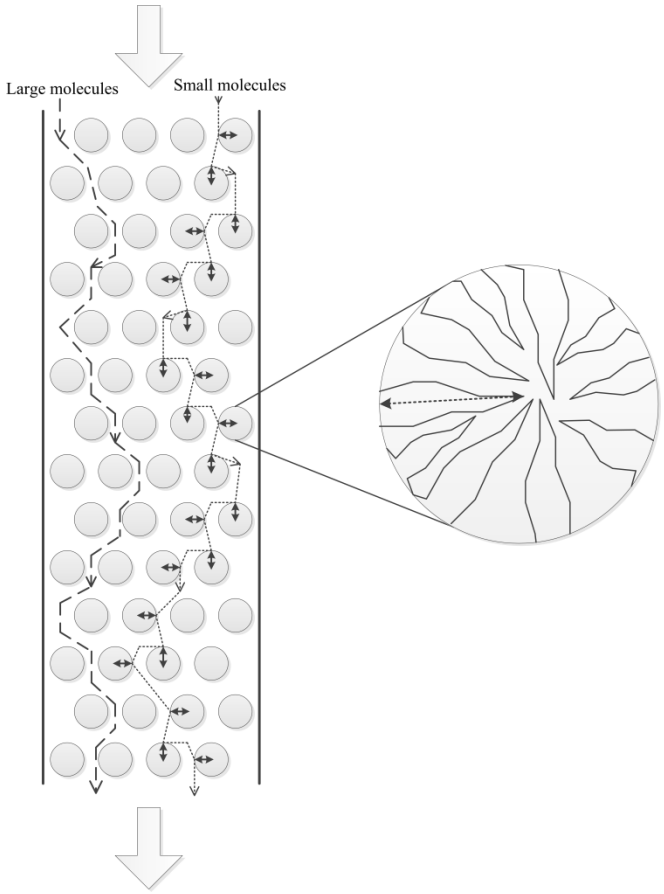

Figure 2.4: The figure shows how molecules with different sizes flow through the size exclusion column. Large molecules avoid the particles and smaller molecules diffuse in to the pores of the particles.

2.4 PEGylation reaction

The PEGylation reaction is a covalent attachment of PEG to a protein or a peptide. The most common reaction is between PEG and an active N-terminal amino group (Figure 2.5). Example of some amino acids which is used in the PEGylation is lysine, arginine, glutamic acid, serine and tyrosine. Lysine is one of the most frequent amino acid in proteins and therefore often chosen to connect with the PEG chains. Lysine also have two possible N-terminals, alpha and epsilon amino groups. Other ways to PEGylate is through the C-terminal carboxylic acid and when using glycoproteins two close hydroxyl groups are oxidized. (7,16,17)

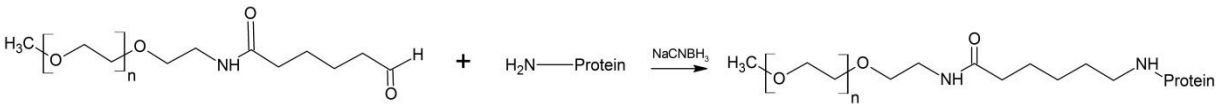

Figure 2.5: This is the main reaction for the PEGylation reaction between a mPEG-aldehyde and the active lysine part of the protein.

Due to the possibility for PEG chains to attach to multiple sites of the protein the finished product could have a mixture of different PEGylated proteins. In the case where lysozyme is PEGylated there are three possible active sites resulting in monoPEGylated, diPEGylated and triPEGylated protein and seven PEGylated configurations excluding the native protein (Figure 2.6). (7)

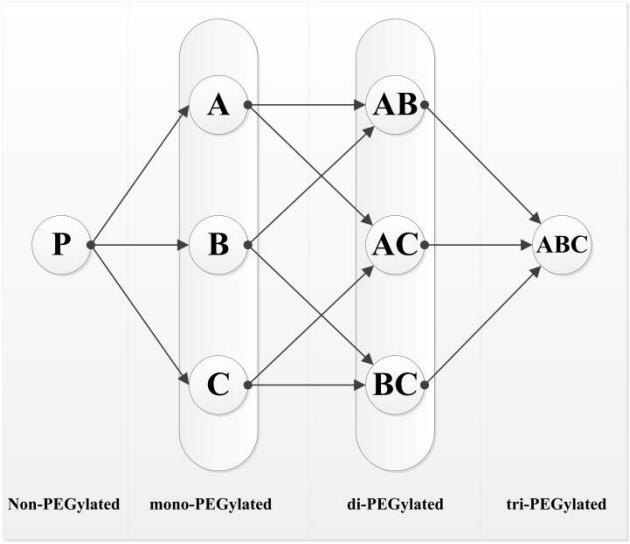

Figure 2.6: In Lysozyme there exist three possible lysine locations for the PEG chain to attach. This gives the multiple reaction pathways to react according to the figure above.

To fully understand the PEGylation reaction a deeper investigation of the reaction mechanism is needed. The reaction mechanism presented below is an example where mPEG-aldehydes are covalently attached to an active N-terminal of lysine and reduced with sodium cyanoborohydride in a light acidic environment. This is one of many possible solutions to successfully PEGylate a protein. (7,16,17)

The first step of the mechanism is that the active ends of the PEG chain take up a positive hydrogen ion (Figure 2.7). The oxygen becomes positive and will draw electrons from the carbon giving the active end a dipole moment. (18,19)

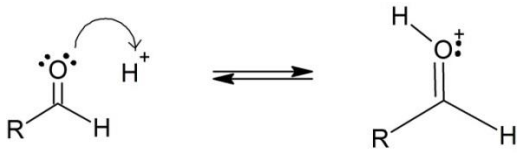

Figure 2.7: Step 1 of 8 in the main reaction mechanism.

The second step is a nucleophilic addition where the N-terminal from lysine attacks the carbon (Figure 2.8). The double bond between the carbon and oxygen becomes a single bond and the oxygen charge becomes neutral. This step creates the covalent bond between the PEG chain and Lysozyme. (18,19)

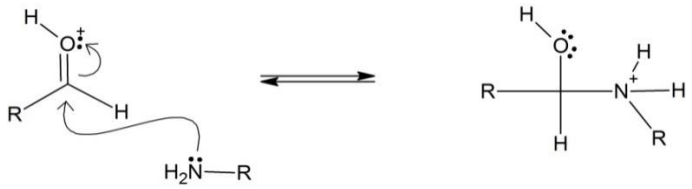

Figure 2.8: The figure describes the second step of the mechanism for the main reaction.

The third step is an acid/base reaction where the base picks up hydrogen from the nitrogen (Figure 2.9). The bond returns to the nitrogen and becomes a free electron pair. (18,19)

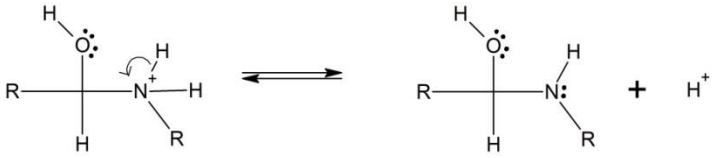

Figure 2.9: The figure shows the third step of the mechanism.

Step four is also an acid/base reaction but this time the base release the hydrogen to the oxygen (Figure 2.10). The oxygen is now carrying two hydrogens and is therefore positively charged. (18,19)

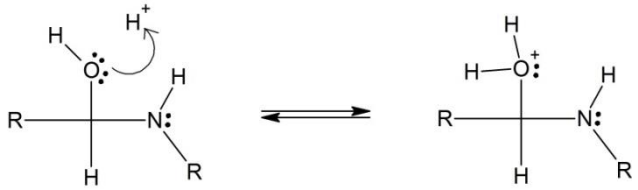

Figure 2.10: Step 4 of 8 in the main reaction mechanism.

In the fifth step the bond between carbon and oxygen is released and the molecule becomes dehydrated. At the same time the carbon connects the nitrogen with a second bond by the help of the free electron pair from the nitrogen (Figure 2.11). (18,19)

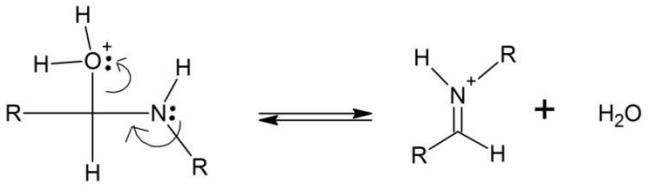

Figure 2.11: The figure illustrates the fifth mechanism step of the main reaction.

The sixth step of the mechanism is the last reversible reaction step. Here the acid catalyst returns to its original state. A water molecule attacks and takes up the last hydrogen (Figure 2.12) connected to the nitrogen and therefore neutralizing the positive nitrogen atom. (18,19)

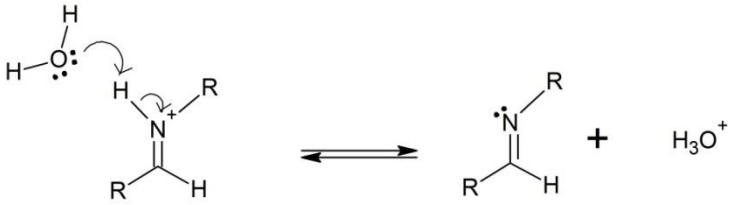

Figure 2.12: The figure displays the mechanism step 6 of 8 for the main reaction.

Step seven is the first irreversible reaction and the step where sodium cyanoborohydride is consumed. Sodium cyanoborohydride reduces the carbon atom with hydrogen (Figure 2.13) and the double bond decreases to a single bond giving the nitrogen a negative charge. This step is assumed to be the rate-determining step of the PEGylation reaction. (18,19)

Figure 2.13: The mechanism step 7 of 8. It's the first irreversible step of the main reaction and also the first step where the reducing agent is used.

The last step of the mechanism is also an irreversible step. The negative charge of the nitrogen atom is neutralized with a hydrogen ion (Figure 2.14) and the PEGylation is now completed. (18,19)

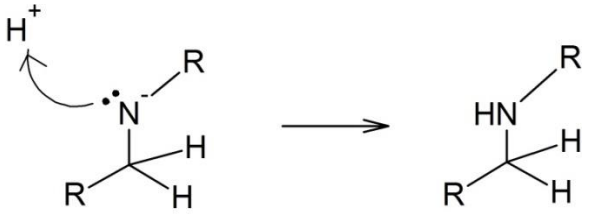

Figure 2.14: The last step of the main reaction mechanism. This step is also an irreversible reaction.

At the same time as the PEGylation reaction, a deactivation of the PEG chain occurs. Sodium cyanoborohydride have the possibility to reduce the active end in the PEG chains. This is done in the same way as step seven and eight of the PEGylation mechanism (Figure 2.15). (18,19)

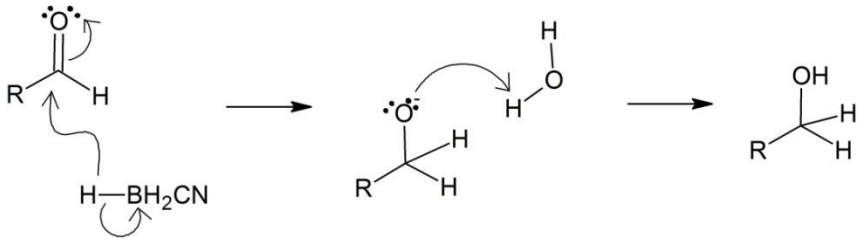

Figure 2.15: The figure illustrates the 2-step deactivation reaction between the PEG molecule and the reducing agent making the PEG chain unavalible for the PEGylation reaction.

2.5 PEGylation processes

2.5.1 Batch reactor process

The most frequently used PEGylation process is conducted with a batch reactor. The batch reactor is preferred due to the often slow reaction kinetics. The batch reactor gives a tradeoff whether the user wants a high yield or low concentration of byproducts. A low reaction time offers low byproduct (multiPEGylated protein) concentration but the conversions of protein to monoPEGylated protein are also low and thus achieve a high amount of unreacted pharmaceuticals that will be wasted. (8,20,21)

After the batch reactor, the products and remaining reactants are separated, either with an ion exchange chromatography (IEC) column or with a size exclusion chromatography (SEC) column. The benefits with using IEC are that it is easy to clean, have a higher capacity and they are smaller than a SEC and thereby making it possible to achieve higher flow rates without high pressure drops. A SEC column on the other hand does not need to use high salt concentrations. (8,20)

2.5.2 Combined recirculation process

To be able to reach a higher yield without producing a large amount of byproducts a recirculation of unreacted protein is sent back to the batch reactor. This makes it possible to stop the batch reaction earlier with the result of a low byproduct concentration. The unreacted protein is then recycled back to the batch reactor where it can PEGylate again resulting in a higher monoPEGylated protein yield. The number of recirculations is however restricted because of an unwanted side reaction where the protein slowly forms a degraded version of itself. (21,22)

2.5.3 Size exclusion reaction chromatography process

The new interesting process which is still under development is size exclusion reaction chromatography (SERC). This method combines reaction and separation in a SEC column. It is therefore possible to remove the monoPEGylated protein before it PEGylates another time resulting in a higher selectivity for the process. (5,23)

The process can be performed in two different ways. The first one (Figure 2.16) have a load with both reactants. The reactants also have approximately the same molecular weight and therefore will the reaction zone move all the way through the column. The product, that will have higher molecular weight, will go faster through the column and thereby leaving the reaction zone. (5,23)

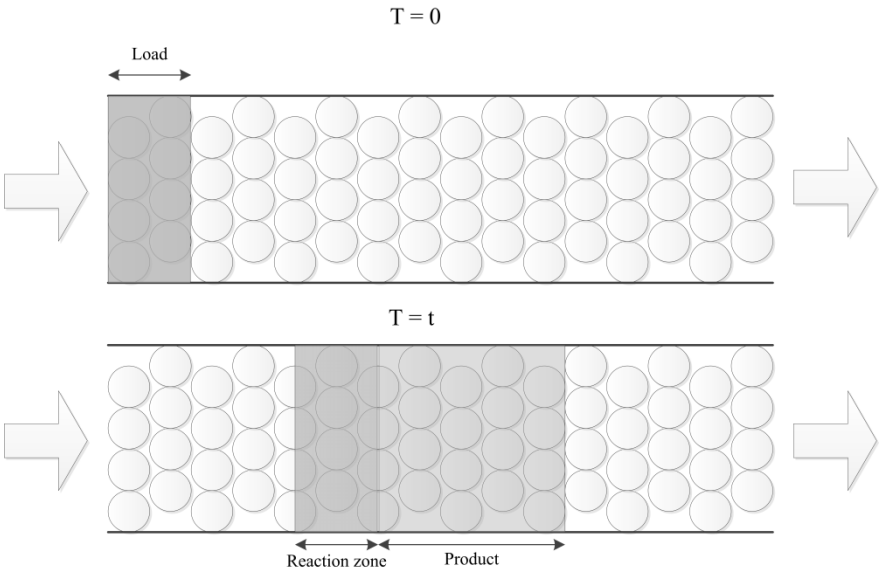

Figure 2.16: The figure illustrates how the SERC column produces and separates the product at the same time.

The second version of the process (Figure 2.17) is where the reactants have different molecular weights and therefore moving at different velocities through the column. The load is then divided into two, where the reactant with the low molecular weight is injected first. The second reactant will have a higher molecular weight resulting in a faster flow that will catch up the other reactant. A reaction zone is created during the second loads passing of the first. The product that is created in the reaction zone will have a larger molecular weight than the reactants and therefore achieve a greater speed. In the end it is possible to collect both reactants and the product separated from each other. (5,23)

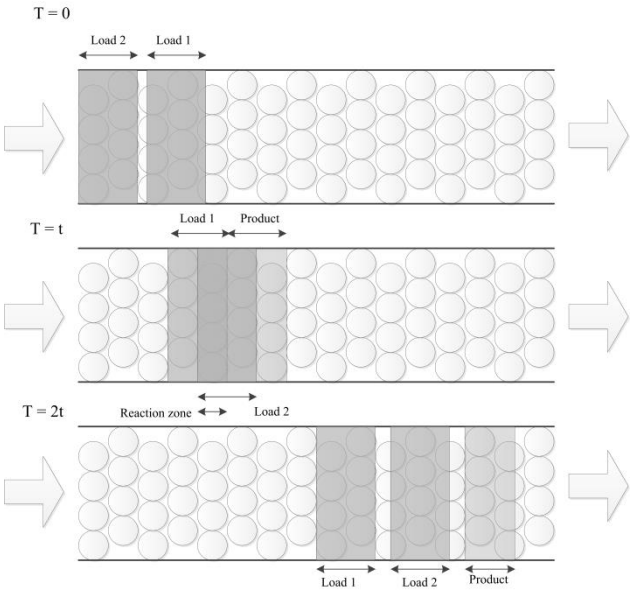

Figure 2.17: The figure shows the SERC column when using two reactants with different molecular weights.

3 Materials & Methods

3.1 Experiments

3.1.1 Materials

All the chemicals were obtained from Sigma-Aldrich (Schnelldorf, Germany). The Superdex 200 10/300 GL SEC column was purchased from GE Healthcare (Uppsala, Sweden). The column was connected to an Äkta purifier system from GE Healthcare (Uppsala, Sweden). A refractive index detector RID-6A from Shimdzu (Kyoto, Japan) was also used.

3.1.2 Preparations

For the Äkta purifier system four solutions were prepared: To clean and preserve the SEC column a solution with 1.0 M NaOH and a solution with 20% ethanol were prepared and degassed. The system was also equipped with degassed distilled water. As a buffer for the column, a degassed solution containing 25 mM sodium phosphate and 150 mM sodium chloride was prepared. The reaction buffer containing 40 mM sodium cyanoborohydride and 50 mM sodium phosphate was prepared and degassed.

3.1.3 Batch reactor

The batch reactor tests were divided into two different experiment types, kinetic and long reaction time. The long reaction time was used to calibrate the SEC column and the kinetic test was used to calibrate the kinetic constants. Each individual test parameters used can be seen in the appendix (Table 10.1).

The long time reaction was mixed with 7.5 ml of Lysozyme (10 mg/ml) and reaction buffer solution together with 7.5 ml of 0.1575 g PEG (6:1 PEG to Lysozyme molar ratio) dissolved in distilled water. The reaction was then carried out in a 20 ml continuous stirred batch reactor and at room temperature. The first sample was taken after 5 min and between one and ten hours of reaction time the samples were taken with an hour interval. The last sample was taken after 24 h. Each sample was a total of 0.50 ml. The samples were directly injected to the SEC column for analysis.

The kinetic experiment was divided into four identical experiments. Reaction buffer and 2.5 ml of Lysozyme (15 mg/ml) were mixed with 0.105 g PEG (8:1 PEG to Lysozyme molar ratio) dissolved in 2.5 ml distilled water. The reactions were carried out in a 7.5 ml continuous stirred batch reactor and at room temperature. A total of ten samples were taken under a three hour reaction time and directly injected into the SEC column for evaluation.

3.1.4 Size exclusion chromatography separations

Column void, particle porosity and dead volume were needed to be tested before the batch reaction samples could be analyzed. For the dead volume test a solution with 1.0 mg/ml Lysozyme was injected to the system without a coupled column. The column void and particle porosity were tested with the injection of a solution with 1.0 mg/ml Blue DEXTRAN 2000 and 0.50 % acetone. All the samples were injected to a 100 µl injection loop and transferred through the column with the flowrate of 0.50 ml/min. The separation was observed and recorded with UV light at a wavelength of 280 nm. The PEG molecules are not visible with UV light and are therefore detected and analyzed with a refractive index detector.

3.1.5 Size exclusion reaction chromatography tests

The SERC experiments were prepared with 1.0 ml of Lysozyme (15 mg/ml) and reaction buffer solution mixed together with 0.0420 g PEG dissolved in 1.0 ml distilled water. The prepared solution was injected into the SEC column instantly after mixing. The injection loop had a volume capacity of 500 µl. The sample flowed through the column with the speed of 0.30 ml/min. The reaction and separation were observed and recorded in the end of the column with UV light at a wavelength of 280 nm. Each experiment conducted, can be seen in the appendix (Table 10.2).

3.2 Simulations

All the simulations were programmed with the computer program Matlab R2013a® (24). To solve the differential equations Matlab®'s built in function *ode15s* was used. For the kinetic calibration another built in function was used: *lsqcurvefit*. When optimizing the process a set of combined functions were used. The foundation used the built in function *fminsearch* and the main function used is *fminsearchbnd* (25).

3.2.1 Batch reaction model

In the simulations of the batch reactor the PEGylation reactions were considered to be of pseudo first order irreversible reaction (Equation 1-3). Sodium cyanoborohydride plays an important role in the time dependent step of the reactions and was therefore included in the reaction equations. The Lysozyme was assumed to be able to PEGylate three times.

$$r_1 = k_1 \cdot c_{lysozyme} \cdot c_{PEG} \cdot c_{CNBH_3} \tag{1}$$

$$r_2 = k_2 \cdot c_{monoPEG} \cdot c_{PEG} \cdot c_{CNBH_3} \tag{2}$$

$$r_3 = k_3 \cdot c_{diPEG} \cdot c_{PEG} \cdot c_{CNBH_3} \tag{3}$$

The simulation also has to take into account the deactivation reaction between the PEG molecule and sodium cyanoborohydride. The deactivation reaction was assumed to be a first order reaction (Equation 4).

$$r_4 = k_4 \cdot c_{PEG} \cdot c_{CNBH_3} \tag{4}$$

Each substance depends on the reactions according to Equations 5-9. Sodium cyanoborohydride was calculated in the same way as PEG (Equation 9), because both were assumed to be consumed in all reactions.

$$\frac{\partial c_{lysozyme}}{\partial t} = -r_1 \,, \frac{\partial c_{monoPEG}}{\partial t} = r_1 - r_2 \,, \frac{\partial c_{diPEG}}{\partial t} = r_2 - r_3 \,, \frac{\partial c_{triPEG}}{\partial t} = r_3 \,, \frac{\partial c_{PEG}}{\partial t} = -\sum_{i=1}^{4} r_i \tag{5-9}$$

3.2.2 Size exclusion reaction chromatography model

The SERC column was simulated using the General rate model. Each substance was individually calculated to flow through the column void according to Equation 10. The equation takes into consideration that the concentrations were dependent on dispersion, convection, pore diffusion and reaction along the column length.

$$\frac{\partial c_{b,i}}{\partial t} = D_{ax}\frac{\partial^2 c_{b,i}}{\partial z^2} - \frac{v}{\varepsilon_c}\frac{\partial c_{b,i}}{\partial z} - \frac{1-\varepsilon_c}{\varepsilon_c}\frac{3}{r}k_f\left(c_{b,i} - c_{p,i}\right) + r_{b,i} \tag{10}$$

The dispersion, convection and reaction inside the pores were calculated individually for each substance with Equation 11. Equation 11 was simulated simultaneously with Equation 10.

$$\frac{\partial c_{p,i}}{\partial t} = \frac{D_e}{\varepsilon_p \cdot K_{D,i}}\left(\frac{\partial^2 c_{p,i}}{\partial r^2} + \frac{2}{r}\frac{\partial c_{p,i}}{\partial r}\right) + r_{p,i} \tag{11}$$

The column starts the simulation without any substances inside (empty column). This was described with the initial conditions from Equation 12 for the void and Equation 13 for the pores.

$$c_{b,i}(t=0,z) = 0 \quad , \qquad c_{p,i}(t=0,r) = 0 \tag{12, 13}$$

The boundary condition for the beginning of the column was described with a Dirichlet condition (Equation 14). The end of the column was described with a von Neumann boundary condition (Equation 15).

$$c_{b,i}(t, z=0) = c_{in,i}(t) \quad , \qquad c_{b,i}(t, z=L) = 0 \tag{14, 15}$$

The diffusion into the pore can be defined as a Robin boundary condition (Equation 16). In the middle of the pore it is assumed no flux which was described with a von Neumann condition (Equation 17).

$$k_f\left(c_{b,i} - c_{p,i}\right) = D_e\left.\frac{\partial c_{p,i}}{\partial r}\right|_{r=R} \quad , \qquad D_e\left.\frac{\partial c_{p,i}}{\partial r}\right|_{r=0} = 0 \tag{16, 17}$$

The dispersion coefficient for the column void was calculated using the Peclét correlation (Equation 18) (26). The Peclét number was assumed to be equal to 1.0.

$$D_{ax} = \frac{v \cdot d_p}{Pe} \tag{18}$$

Equation 19, also called the Wilson-Geankopolis correlation, was used to calculate the mass transfer coefficient (26). The free diffusivity coefficient was approximated to 10^{-6} for all components.

$$k_f = 1.09\frac{v^{1/3}}{\varepsilon_c}\left(\frac{D_M}{2r}\right)^{2/3} \tag{19}$$

The partial differential equations were solved by using the Method of lines (MOL)(27) method that discretized the column in space. The derivatives inside each element were approximated with the Finite volume method (FVM) (28). The column was divided into a mesh of 300 grid points and the particles used a mesh of 10. The dispersion was approximated with a 3 point central approximation (Equation 20) for both the column and the particle equations. The

convection in the pore also used a 3 point central approximation (Equation 21). The convection in the column used a flux limiter that combined the 3 point central approximation (Equation 21) with a 2 point backward approximation (Equation 22) to avoid oscillation effects.

$$\frac{\partial^2 c_n}{\partial z^2} = \frac{c_{n+1} - 2c_n + c_{n-1}}{h^2} \quad , \quad \frac{\partial c_n}{\partial z} = \frac{c_{n+1} - c_{n-1}}{2h} \quad , \quad \frac{\partial c_n}{\partial z} = \frac{c_n - c_{n-1}}{h} \tag{20-22}$$

The flux limiter was applied according to Equation 23. When the flux derivatives in column were high, a 2 point backward approximation was dominant and when the flux derivatives were low, a 3 point central approximation was dominant.

$$F(c_n) = [f_{n+1/2}^{low} - \phi(s_n)(f_{n+1/2}^{low} - f_{n-1/2}^{high})] - [f_{n-1/2}^{low} - \phi(s_{n-1})(f_{n-1/2}^{low} - f_{n-1/2}^{high})] \tag{23}$$

The flux limiter function ϕ(s) was calculated with Equation 24 which is the van Albada symmetric limiter function. The ϕ(s) function was restricted to only positive values; negative values were automatically assigned the value zero. s was described as the ratio of sequential gradients in the mesh (Equation 25). A small number was added to avoid the possibility of division by zero.

$$\phi(s) = \frac{s^2 + s}{s^2 + 1} \quad , \quad s_n = \frac{c_n - c_{n-1}}{c_{n+1} - c_n + 10^{-16}} \tag{24, 25}$$

3.2.3 Calibrations

The calibration of the kinetic constants was done with the experimental data from batch reactor test P. The built in function *lsqcurvefit* used the batch reaction model to optimize the four kinetic constants in order that the simulations matched the experimental data. *Lsqcurvefit* was given the following inputs: the function for the batch reaction model, the guessed values for each constant, the time and concentrations from the experiments and a lower boundary for each constant. The lower boundary was used to ensure that the kinetic constants were assigned a positive value.

The partition coefficient for each PEGylated substance, native protein and PEG were calibrated with Equation 26. The retention volumes for the substances were calculated using the average peak maximum from the batch experiment H (Table 10.1 in Appendix) chromatography results. The partition coefficient for the PEG molecule was calibrated with the results from the refraction index data. The reducing agent was assumed to access the total pore volume.

$$K_{D,i} = \frac{V_i - V_c}{V_t - V_c} \tag{26}$$

3.2.4 Optimizations

The optimization of the PEGylation process was done by using the SERC model to calculate the amount monoPEGylated protein and native protein at the end of the column. The native protein was assumed to be totally separated from the monoPEGylated protein. The native protein was after the column recirculated back to the SERC column for another PEGylation run. The SERC model runs a total of ten times (nine recirculations). The optimization was limited by alter two parameters together with the best amount of recirculation steps according to the calculated object. The parameters altered were the flow through the column (max 0.75

ml/min, min 0.25 ml/min) and the PEG to Lysozyme molar ratio (max 12, min 1). The boundaries for the parameters were set according to the regulations from the manual for the SEC column. The objective for the optimization was calculated with multiple equations to get an overview of possible solutions.

The first objective was calculated with Equation 27. The objective was to minimize the amount of PEG molecules needed to produce one monoPEG molecule. The equation avoids the possibility of achieving the result with no production by dividing the mole fraction of monoPEG with the so called total PEG to monoPEG molar ratio.

$$objective = -\frac{x_{monoPEG}}{(\sum n_{PEG})(n_{monoPEG})^{-1}} \tag{27}$$

Equation 28 was used to calculate the second objective. This objective was created to maximize the productivity of monoPEGylated protein.

$$objective = -\frac{x_{monoPEG}}{t} \tag{28}$$

The objective calculated with Equation 29 was able to minimize the waste of native protein.

$$objective = -\frac{1}{x_{Lysozme}} \tag{29}$$

In Equation 30 the objective calculated the maximum monoPEG production possible.

$$objective = -x_{monoPEG} \tag{30}$$

To avoid the production of multiPEGylated protein the Equation 31 was implemented. The production of monoPEG was also added to the equation in the same way as Equation 27 to avoid zero production.

$$objective = -\frac{x_{monoPEG}}{x_{multiPEG}} \tag{31}$$

The objective in Equation 32 is a combination of productivity, minimize PEG usage and minimize the waste of native protein.

$$objective = -\frac{x_{monoPEG}}{t} \cdot \frac{x_{monoPEG}}{\sum n_{PEG} / n_{monoPEG}} \cdot \frac{1}{x_{Lysozyme}} \tag{32}$$

Equation 33 calculated the objective when combining maximum monoPEG production with productivity.

$$objective = -x_{monoPEG} \frac{x_{monoPEG}}{t} \tag{33}$$

4 Results

4.1 Experimental results

The results from the batch reaction show that a fast reaction between Lysozyme and mPEG-propionaldehyde can be achieved with the concentrations of 7.5 mg/ml Lysozyme, 20 mM sodium cyanoborohydride and a 8:1 PEG to Lysozyme molar ratio. The long time reaction results confirm that Lysozyme has the possibility of PEGylate three times (Figure 4.1).

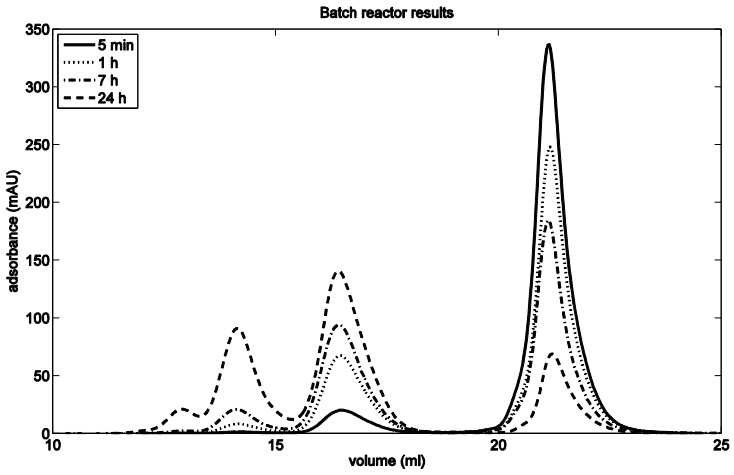

Figure 4.1: The figure shows the change of concentrations in the batch reactor. The peaks with the start from right are: Lysozyme, monoPEG, diPEG and triPEG.

The SERC experiments confirm the possibility to conduct a reaction inside the SEC column. The separation between native and monoPEGylated protein makes it possible to pool both with a yield and purity over 95 %. The reaction in the SERC column is confirmed more selective in comparison to the batch reaction under these conditions. The same amount of produced monoPEG in the batch reactor as the SERC column accumulates more multiPEGylated protein.

4.2 Calibration results

The calibration results for the kinetic constants can be seen in Table 4.1. The constants define a decreasing kinetic velocity for each PEGylation of the protein which can be described by that the protein becomes less available for the PEG chains in every PEGylation step.

Table 4.1: The table illustrates the calibrated kinetic constants for the four reactions.

Constant	Value
$\mathbf{k_1}$	5.74 $dm^6/mole^2s$
$\mathbf{k_2}$	3.96 $dm^6/mole^2s$
$\mathbf{k_3}$	3.47 $dm^6/mole^2s$
$\mathbf{k_4}$	0.0282 $dm^3/moles$

The results from the calibration of the partition coefficients can be seen in Table 4.2. The refraction index experiment confirms that the PEG chains have access to the total pore volume.

Table 4.2: the table shows the calibrated porosity constants for each substance.

Substance	K_D
Lysozyme	0.978
MonoPEG	0.626
DiPEG	0.454
TriPEG	0.369
PEG	1.00

4.3 Validation

4.3.1 Kinetics

The validation of the kinetic constants is showed in Figure 4.2. The model has a good fit against the experimental data. The experimental data is taken from batch reactions P (four identical experiments).

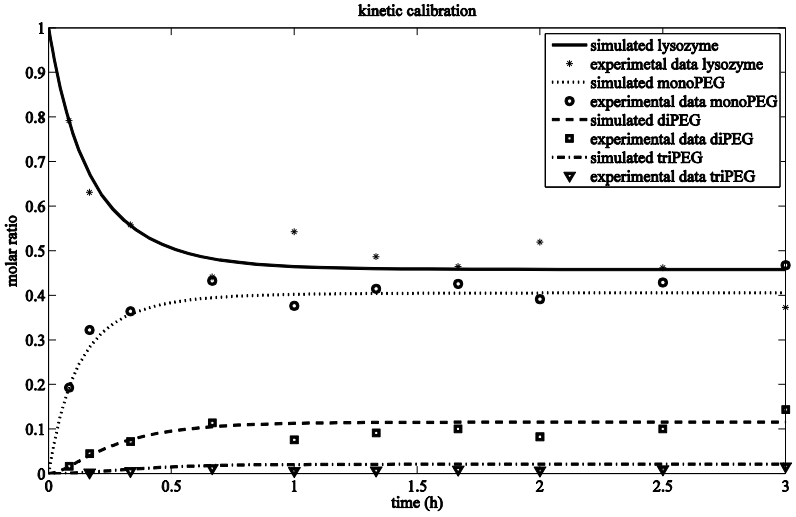

Figure 4.2: The figure illustrates the validation of the kinetic constants.

4.3.2 Size exclusion chromatography

The partition coefficients are validated with Figure 4.3. The chromatogram used in the figure is taken from the 24 h test from batch reaction experiment H. The peaks were well adapted to the experimental data.

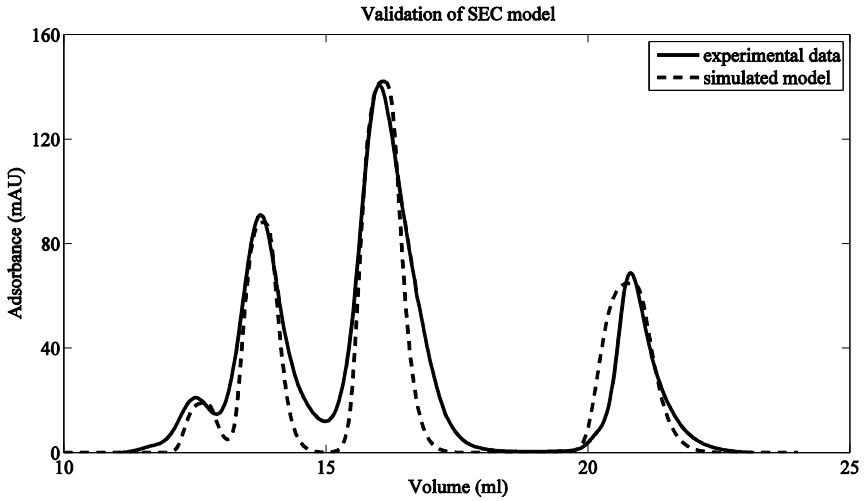

Figure 4.3: The figure illustrates the adaptation of the simulated SEC model against the experimental data, validating the SEC model.

4.3.3 Size exclusion reaction chromatography

The validation of the SERC model is shown in Figure 4.4. The model is considered to have a sufficient adaptation against the experimental data. The model also confirms the reaction due to approximately the same end concentrations.

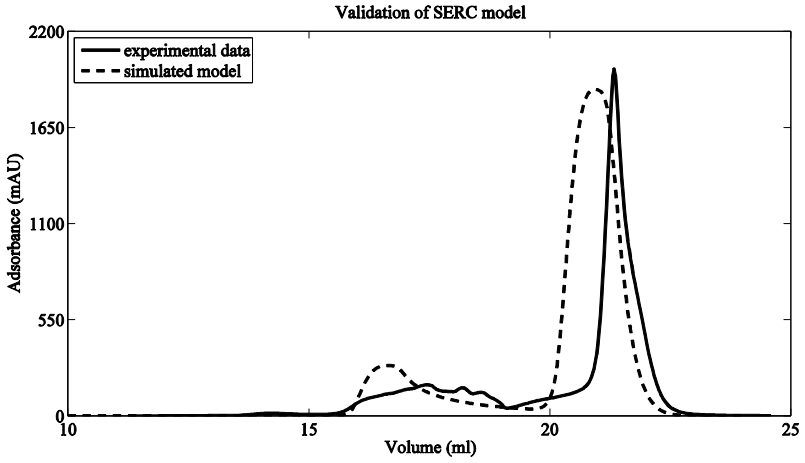

Figure 4.4: The figure shows the adaptation of the simulated SERC model against the experimental data, validating the SERC model. The experimental data is taken from SERC reaction test Q.

4.4 Optimization results

The recirculation of Lysozyme back to the SERC column is shown in Figure 4.5. The simulation also confirms a greater selectivity than the batch reaction. When recirculating more than two times an increase in yield compared to the batch reaction is achieved.

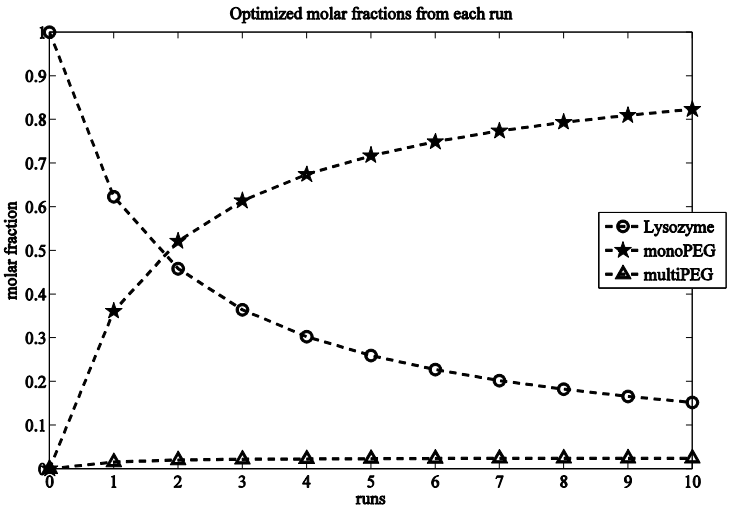

Figure 4.5: The figure illustrates total molar fraction Lysozyme, monoPEG and multiPEG after each recirculation cycle.

The optimization simulations reach a possible monoPEG yield up to 82.3 % after the ninth recirculation (objective equation 29). The simulations also report a maximum multiPEG yield of 2.46 %. It can also be shown that it requires a minimum of 41 PEG molecules to produce one monoPEGylated protein molecule (objective equation 27). For all optimizations the Lysozyme concentration is fixed at 10 mg/ml. The results from the different optimizations can be seen in Table 4.3.

Table 4.3: The table shows the results from the optimization according to referred equation in chapter 3.2.4. The Lysozyme concentration is fixed at 10 mg/ml.

Objective equation	**Flow (ml/min)**	**PEG: Lysozyme ratio**	**PEG: monoPEG ratio**	**Recirculation**	**MonoPEG (%)**	**MultiPEG (%)**	**t (h)**
27	0.25	5.02	41.0	9	68.4	0.866	16.0
28	0.75	12	60.5	0	19.8	0.320	0.533
29	0.25	12	55.2	9	82.3	2.46	16.0
30	0.25	12	55.2	9	82.3	2.46	16.0
31	0.75	1	55.8	9	16.5	0.0203	5.33
32	0.25	12	43.5	3	67.4	2.31	6.40
33	0.75	12	73.8	3	49.3	0.654	2.13

Figure 4.6 is used to validate the optimization for equation 33. Each surface represents the objective value for different flowrates and PEG to Lysozyme molar ratios when recirculated a specific number of steps.

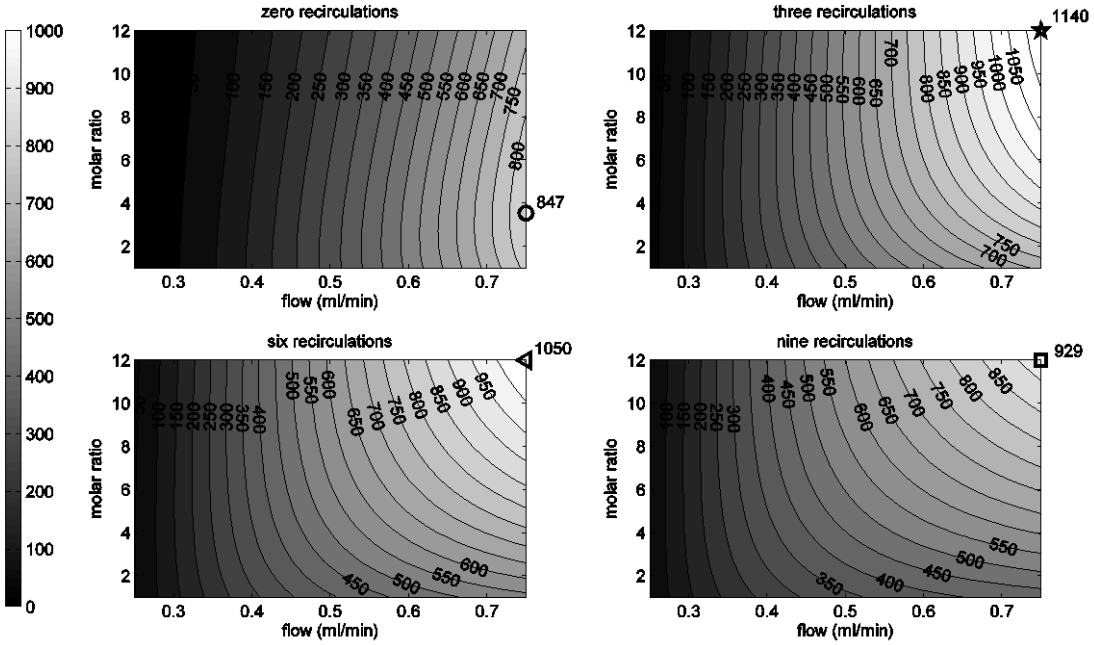

Figure 4.6: The figure shows the optimization when lysozyme has been recirculated zero, three, six and nine times. The objective is calculated with equation 33 where three recirculation steps achieve the highest value. The star represents the optimum for three recirculation steps, the circle represents zero recirculation steps, the triangle represents six recirculation steps and the square represents nine recirculation steps.

5 Discussion

5.1 Experiments

5.1.1 Batch reactor

The kinetic reaction was divided into four experiments. A better result would have been achieved if all the samples came from the same experiment. This would be possible if the system could have been running multiple SEC columns at the same time or something that could have stopped the reaction from taking place in the sample. A test where a small amount of sodium hydroxide was added to the sample was performed unsuccessfully.

Another test where the parameters were altered should have been done to further validate the kinetic constants. The PEGylated proteins were detected with an UV light at a wavelength of 280 nm. It is also possible to use wavelengths at 254 nm and 215 nm at the same time to get more accurate results.

5.1.2 Size exclusion reaction chromatography

The best results from the SERC column were given when the reactants were mixed in the exact moment they entered the column. If the reactants are injected manually (done in the experiments) there will be a residence time up to two minutes where the reactants can react like in the batch reactor. This will result in a higher final concentration for both the monoPEGylated protein and the multiPEGylated protein.

5.2 Simulations

5.2.1 Models

The batch reactor model assumed four irreversible reactions. This is only validated under a three hour period. The 24 hour experiment shows that a reaction still occurs after it theoretically should have stopped. It is possible that the deactivation reaction of the PEG molecule is a reversible reaction.

Both the SEC model and the SERC model use the general rate model to describe the concentrations inside the column. It is also possible to use the dispersion model. The dispersion model describes a total void volume accessible for the specific molecule instead of describing the diffusion into the pores. In the SEC model case it is possible that the dispersion model is a good approximation but in the SERC model case the reactions need more accurate concentrations both in the void and pores.

The Peclét number, diffusion coefficient and free diffusion coefficient are coefficients that have been given an assumed value. All the coefficients are also assumed to be the same value for all the substances. The Peclét number can be calibrated with experimental data but it is often given a standard value used for macromolecules in packed beds. It is possible to calculate the free diffusion coefficient with the Stoke-Einstein equation or calibrate the value from experimental data. Both ways will give specific values for each substance. The diffusion coefficient can also be calibrated from experimental data or estimated with equations.

As mentioned before a mixing of the reactants were done before the injection. The SERC model does not simulate the reactions that occur before the column. This can be solved with either introducing a small batch reactor with a residence time of approximately two minutes before the column in the simulations or making an automatic injection loop that have the possibility of injecting the reactants at the same time to the column in the experiment.

5.2.2 Optimization

The optimization applies the assumption that the monoPEGylated protein is fully separated from the native protein. The simulation of each recirculation step assumes that the unPEGylated protein can be recirculated and purified to match the same load volume as the first step. The steps after the column need a separation step where the unPEGylated protein is separated from the PEG chains and a purification step where the unPEGylated protein is collected in a total volume matching the original load.

The optimization uses seven different object equations, each with its own purpose as described in the method. The cost objective is one objective that is the most important and not in this report. The experiments use Lysozyme (a cheap protein) and a PEG-chain that in comparison to the Lysozyme is an expensive molecule. In real cases is the protein (pharmaceutical) the expensive molecule and the PEG-chain is possibly the cheap molecule. This makes it impossible to estimate a good cost objective with the available data. A possible solution is to use a cost ratio between the molecules.

5.2.3 Restrictions

The simulations are restricted by experimental boundaries like viscosity and SEC column limits. Lysozyme and the PEG-chain are both large molecules that generate high viscosities when increasing the concentrations. The SEC column limits the protein concentration to 10 mg/ml when using a load of 0.5 ml. The PEG chain concentration is limited to a PEG to Lysozyme molar ratio of 12:1. This limit is chosen because of the extrapolation from the experimental validation. The column flow is restricted to a minimum of 0.25 ml/min and a maximum of 0.75 ml/min.

6 Conclusions

The experiments confirm that Lysozyme is able to PEGylate three times. The reactions achieve a velocity approved for experiments with size exclusion reaction chromatography. The batch reaction experiment validates the batch reactor model with three PEGylation reactions and one deactivation reaction.

The SERC experiment confirms and validates that a reaction can occur inside a SEC column as well as improving the reaction selectivity. The best experimental result of monoPEGylated protein yield was achieved with a flow at 0.3 ml/min and a load volume of 0.5 ml.

The calibration of the kinetic constants achieved values of 5.74, 3.96 and 3.47 ($dm^6/mole^2s$) for the reactions into monoPEGylation, diPEGylation and triPEGylation respectively. The kinetic constant of the deactivation reaction was calibrated to 0.0282 ($dm^3/moles$). In the SEC column the PEG chains used 100 % of the particle pores. The native, monoPEGylated, diPEGylated and triPEGylated protein used 97.8 %, 62.6 %, 45.4 % and 36.9 % respectively of the particle pores.

The optimization shows that it is potential to reach a yield of 82.3 % monoPEGylated protein when recirculating the unPEGylated protein nine times. In comparison to the batch reaction yield of approximately 40 % monoPEGylated protein the SERC column with recirculation have a possibility of doubling the production yield. The amount of multiPEGylated protein reaches a maximum yield of 2.46 %, thereby improving the selectivity substantially.

7 Future Work

Future work with an automatized injection where the protein and PEG chain are mixed at the same time as they are entering the column could be able to improve the SERC column experiments so that reactions before the column are avoided.

This work only simulates the SERC column which means that more work about the recirculation loop is needed. Is it possible to extract all the unPEGylated protein and purify it to the correct volume? An easy possible solution could be to pool the unPEGylated protein which also includes deactivated PEG molecules and run it through an ion exchange chromatography column. A purifying step with ultra-filtration after the IEC column is used to lower the total volume into the original load volume.

The experiments use a combination of Lysozyme and mPEG-propionaldehyde with a molecular weight of 5000 Da. Next step is to use already FDA approved PEGylated pharmaceuticals to evaluate the existing process.

8 Reference list

1. Abuchowski A, McCoy JR, Palczuk NC, van Es T, Davis FF. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. J Biol Chem. 1977;252(11):3582–6.

2. Fee CJ, Van Alstine JM. Prediction of the viscosity radius and the size exclusion chromatography behavior of PEGylated proteins. Bioconjug Chem. 2004;15:1304–13.

3. Pasut G, Veronese FM. State of the art in PEGylation: The great versatility achieved after forty years of research. J Control Release [Internet]. Elsevier B.V.; 2012;161(2):461–72. Available from: http://dx.doi.org/10.1016/j.jconrel.2011.10.037

4. Yoshimoto N, Isakari Y, Itoh D, Yamamoto S. PEG chain length impacts yield of solid-phase protein PEGylation and efficiency of PEGylated protein separation by ion-exchange chromatography: Insights of mechanistic models. Biotechnol J. 2013;8:801–10.

5. Fee CJ. Size-exclusion reaction chromatography (SERC): A new technique for protein PEGylation. Biotechnol Bioeng. 2003;82:200–6.

6. Maiser B, Kröner F, Dismer F, Brenner-Weiß G, Hubbuch J. Isoform separation and binding site determination of mono-PEGylated lysozyme with pH gradient chromatography. J Chromatogr A [Internet]. Elsevier B.V.; 2012;1268:102–8. Available from: http://dx.doi.org/10.1016/j.chroma.2012.10.047

7. Roberts MJ, Bentley MD, Harris JM. Chemistry for peptide and protein PEGylation. Adv Drug Deliv Rev. 2012;64:116–27.

8. Fee CJ, Van Alstine JM. PEG-proteins: Reaction engineering and separation issues. Chem Eng Sci. 2006;61:934–9.

9. Harris JM, Struck EC, Case MG, Paley MS, Yalpani M, Van Alstine JM, et al. Synthesis and characterization of poly(ethylene glycol) derivatives. J Polym Sci Polym Chem Ed [Internet]. 1984;22:341–52. Available from: http://dx.doi.org/10.1002/pol.1984.170220207

10. Veronese FM, Pasut G. PEGylation, successful approach to drug delivery. Drug Discov Today. 2005;10(21):1451–8.

11. Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. Nat Rev Drug Discov. 2003;2(March):214–21.

12. Lane CF. Sodium Cyanoborohydride - A Highly Selective Reducing Agent for Organic Functional Groups. Synthesis. 1975. p. 135–46.

13. Ambrogelly A, Cutler C, Paporello B. Screening of reducing agents for the pegylation of recombinant human IL-10. Protein J. 2013;32:337–42.

14. Harris DC. Quantitative chemical analysis. 8th ed. New York: Clancy Marshall; 2010. 719 p.

15. Fee CJ, Alstine JM Van. Separation of PEGylated Proteins by Size Exclusion Chromatography: Influence of PEGylation on Molecular Size. APCChE Conf. 2004;(9085):1–10.

16. Kinstler OB, Brems DN, Lauren SL, Paige AG, Hamburger JB, Treuheit MJ. Characterization and stability of N-terminally PEGylated rhG-CSF. Pharmaceutical Research. 1996. p. 996–1002.

17. Kinstler O, Molineux G, Treuheit M, Ladd D, Gegg C. Mono-N-terminal poly(ethylene glycol)-protein conjugates. Adv Drug Deliv Rev. 2002;54:477–85.

18. Ellervik U, Sterner O. Organisk kemi. 2:2 ed. 2007.

19. Carey AF. Organic Chemistry. seventh ed. McGraw-Hill; 2007. 1312 p.

20. Pfister D, Morbidelli M. Process for protein PEGylation. J Control Release. 2014;180(1):134–49.

21. Otero B, Degerman M, Hansen TB, Hansen EB, Nilsson B. Model-based design and integration of a two-step biopharmaceutical production process. Bioprocess Biosyst Eng. 2014;1989–96.

22. Westerberg K, Broberg-Hansen E, Sejergaard L, Nilsson B. Model-based risk analysis of coupled process steps. Biotechnol Bioeng. 2013;110(9):2462–70.

23. Fee CJ. Reaction process. United States; US 20060128945, 2006.

24. MathWorks. MATLAB R2013a. Natick, Massachusetts, United States: The MathWorks; 2013.

25. D'Errico J. fminsearchbnd for use with Matlab. The MathWorks; 2006.

26. Borg N, Brodsky Y, Moscariello J, Vunnum S, Vedantham G, Westerberg K, et al. Modeling and robust pooling design of a preparative cation-exchange chromatography step for purification of monoclonal antibody monomer from aggregates. J Chromatogr A [Internet]. Elsevier B.V.; 2014;1359:170–81. Available from: http://dx.doi.org/10.1016/j.chroma.2014.07.041

27. Davis ME. Numerical methods and modeling for chemical engineers. Dover ed. New York; 1984.

28. Eymard R, Gallouët T, Herbin R. Finite volume methods. Handb Numer Anal [Internet]. 2000;M(January). Available from: http://www.math.uni.wroc.pl/~olech/courses/skrypt_Roberta_wroclaw.pdf

9 Table of abbreviations

Symbol	Unit	Description
Latin		
c	mole/dm^3	concentration
D_{ax}	m^2/s	dispersion coefficient
D_e	m^2/s	diffusion coefficient
D_M	m^2/s	free diffusion coefficient
d_p	m	particle diameter
F	-	total mesh flux
f	-	flux resolution
h	m	mesh length
i	-	substance index
k	-	kinetic constant
K_D	-	partition coefficient
k_f	m/s	mass transfer coefficient
L	m	column length
n	-	mesh index
n_i	mole	amount of substance
Pe	-	Peclét number
r	m	particle radius
r_b	mole/dm^{3}s	reaction in bulk
r_p	mole/dm^{3}s	reaction in pore
s	-	ratio of successive gradients
t	s	time
v	m/s	velocity
V_c	ml	column void volume
V_i	ml	substance column volume
V_t	ml	column total volume
x	-	molar ratio
z	m	column length
Greek		
ε_c	-	porosity of particle bed
ε_p	-	particle porosity
Φ	-	flux limiter function

10 Appendix

Table 10.1 The table shows the parameters for each test done with the batch reactor.

Experiment	Lysozyme conc. (mg/ml)	PEG molar ratio	PEG type	$NaCNBH_3$ conc. (mM)	Reaction time (h)	Comments
A	5	6:1	PEG-NHS 2000 g/mole	20	3.5	no reaction
B	5	3:1	PEG-NHS 5000 g/mole	20	3	no reaction
C	5	6:1	PEG-NHS 2000 g/mole	20	19.5	no reaction
D	5	6:1	PEG-NHS 2000 g/mole	-	3	pH 8.5 buffer replaces reducing agent, lysozyme conc. decreasing
E	5	10:1	PEG-NHS 5000 g/mole	-	23	pH 8.5 buffer replaces reducing agent, lysozyme conc. decreasing
F	5	6:1	PEG-ald 2000 g/mole	20	5	reaction giving monoPEG and diPEG
G	5	6:1	PEG-ald 5000 g/mole	20	6	reaction giving monoPEG, diPEG and triPEG
H	5	6:1	PEG-ald 5000 g/mole	20	24	used for peak calibration
I	5	6:1	PEG-ald 5000 g/mole	20	5	PEG mixed with reducing agent overnight, lower reaction speed
J	5	6:1	PEG-ald 5000 g/mole	100	2	PEG mixed with reducing agent overnight, no reaction
K	5	6:1	PEG-ald 5000 g/mole	10	5	lower reaction speed
L	5	6:1	PEG-ald 5000 g/mole	40	3	lower reaction speed
M	5	6:1	PEG-ald 5000 g/mole	20	6	confirming reaction G and H
N	10	6:1	PEG-ald 5000 g/mole	20	1	to high concentration
P	7.5	8:1	PEG-ald 5000 g/mole	40	3	test divided into 4 reactions, results used for kinetic calibration

Table 10.2: The table shows the parameters for all the SERC experiments.

Experiment	Lysozyme conc. (mg/ml)	PEG molar ratio	$NaCNBH_3$ conc. (mM)	Flow (ml/min)	Load (ml)	Comments
Q	7.5	8:1	40	0.3	0.5	
R	7.5	8:1	20	0.3	0.5	sampling 1ml from 16-23 ml confirming monoPEG conc.
S1	7.5	8:1	20	0.3	0.1	
S2	7.5	8:1	20	0.3	0.2	
S3	7.5	8:1	20	0.3	0.3	
S4	7.5	8:1	20	0.3	0.4	

Size Exclusion Reaction Chromatography

Den nya tekniken för att förbättra processen av PEGylerade läkemedel

Dagens krav på läkemedel är höga, de ska t.ex. stanna kvar i kroppen under lång tid, ha lång hållbarhetstid och vara lätt att dosera. Att PEGylera ett läkemedel kan ge dessa egenskaper. Idag är dock denna tillverkningsprocess långsam och ger ett lågt utbyte. Size Exclusion Reaction Chromatography (SERC) är en ny teknik som för-hoppningsvis ska förbättra denna process.

Protein är idag vanligt förekommande som läkemedel. De är lätta att producera och kroppen har lätt för att ta åt sig medicinen. Nackdelen med dessa läkemedel är att kroppen med hjälp av bl.a. njurarna kan filtrera bort innan medicinen kan ge full effekt. Proteinen kan även ha korta hållbarhetstider. Detta har forskarna löst genom att koppla på en lång kolkedja till proteinet. Denna kolkedja kallas för Poly-EtylenGlykol (PEG) därav namnet PEGylering för själva processen. PEG tillsammans med proteinet bildar en molekyl som inte filtreras bort av njurarna och därför kan stanna längre i kroppen. Molekylen blir även lättare att lösa upp i vatten samt att den får en bättre hållbarhetstid.

Processen som används idag går ut på att PEG tillsammans med proteinet blandas i en satsreaktor där dessa får reagera under en längre tid. Resultatet av detta är att man får ut en viss del protein som inte har hunnit reagera, en del som har bildat rätt kombination av en PEG-kedja och ett protein. Men det kommer även ut en del protein som kopplats samman med flera PEG-kedjor, så kallat multiPEGylerat protein. Detta innebär att bara ca 60 % av proteinet som tillsätts till reaktorn kan användas som läkemedel. Resten (40 %) går direkt till papperskorgen då dessa molekyler inte är godkända av läkemedels-verket.

För att undvika att få stora delar multiPEGylerat protein kan SERC-processen utnyttjas. SERC-processen använder sig av molekylernas storlek för att separera produkten innan en ny PEG-kedja kan kopplas ihop. En förstorad SERC-kolonn kan beskrivas som en cylinder fylld med innebandy-bollar. Atomer representeras som sandkorn och molekyler t.ex. PEG och protein som mindre stenar. Stora molekyler som PEGylerade protein kan ses som stora stenar. Sandkorn och mindre stenar kan utan problem ta sig in i bollarnas hål. Reaktionen mellan PEG-kedjan och protein innebär att två små stenar bildar en stor. Eftersom sandkorn och små stenar kan röra sig fritt i cylindern tar dessa lång tid att ta sig igenom cylindern. De stora stenarna däremot kommer inte in i bollarna och kan därför bara röra sig mellan dessa. I och med detta kommer stora stenar att ta sig igenom cylindern mycket snabbare.

PEG-kedjan tillsammans med proteinet kan under en längre tid reagera inne i SERC-kolonnen samtidigt som de transporteras genom kolonnen. Produkten monoPEGylerat protein kan tack vare sin stora storlek förflytta sig snabbare genom kolonnen, och därmed undvika att PEGyleras en gång till.

Resultatet av denna nya metod blir en process som inte bara har möjligheten att minska mängden multiPEGylerade protein, utan även öka mängden användbart läkemedel till ca 80 %. Till skillnad från en satsreaktor som behöver rena produkten från övriga ingredienser efter reaktionen, så sker detta redan inuti själva SERC-kolonnen.